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MINIMUM PHOSPHATE REQUIREMENT OF POTATO PLANTS GROWN IN SOLUTION CULTURES[1]

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INTRODUCTION

The determination of the minimum amounts of different nutrients required for the optimum growth of various crop plants has been the subject of considerable research. Much of this has been conducted specifically to determine the minimum phosphorus requirements of certain plants. Extensive studies of this type have been conducted by Parker (*17*),[2] Tidmore (*24*), and Sommer (*22*) on various plants, including corn, cotton, tomatoes, and buckwheat, growing in solution cultures; but practically no work of a similar nature has been attempted on potatoes. The present research was undertaken to determine the minimum level of phosphate (PO_4) required by potato plants as indicated by their growth responses in solution cultures and to study the effect of the supply of phosphate on the amount of phosphorus absorbed and its distribution within the plant.

In a critical study of the minimum phosphate requirements of potato plants low concentrations necessarily must be employed if the amount supplied is to be kept at all representative of soil conditions. This fact has been established through the analyses of soil solutions by Burd and Martin (*5*) and Parker and Tidmore (*18*), who found that the PO_4 content of the soil solutions from productive soils is of an unusually low order—occasionally as high as 2.5 to 3.0 p. p. m. but more frequently less than 0.1 p. p. m. The ability of plants to absorb phosphate from solutions with PO_4 concentrations similar to those found in the soil solution has been known for many years, as pointed out by Teakle (*23*). Among those cited on this point by Teakle are Birner and Lucanus (*3*) and Beyer (*2*), who, prior to 1870, obtained normal growth of oat plants in well water containing 2 p. p. m. of PO_4, and Schlœsing (*19*) and Kossowitsch (*13*), who obtained similar results about 30 years later. Usually, however, plant growth in solution cultures differs somewhat from that in soil; nevertheless, the mineral absorption and growth capable of being obtained in solution cultures are generally considered satisfactory as a measure of the nutrient responses of plants.

[1] Received for publication June 27, 1946.

[2] Italic numbers in parentheses refer to Literature Cited, p. 17.

MATERIALS AND METHODS

The growing of potatoes in the greenhouse, and in solution cultures in particular, presents some problems seldom encountered with other types of plants. Skinner (*21*), Johnston (*10*), and Newton (*16*) grew potatoes in solution cultures and fully recognized the nature of the problems involved, but they were unable to devise satisfactory equipment or a suitable technique to overcome these problems fully. The ideal equipment for growing potatoes in nutrient solutions should provide for the plant and the tubers a means of support that will allow them to grow above the solution and yet in no way interfere with its nutrient value. Figures 1 and 2 show the type of equipment designed to meet these requirements. The plant is supported directly on a square of ⅛-inch mesh nylon screen [3] tacked in place over a 5-inch opening in the wooden substage. As used in these experiments, nylon proved to be an ideal material for supporting the potato roots and tubers. The same nylon screens were used repeatedly in different experiments, subjected to alternating dry and wet conditions, and even exposed to mold growth without developing signs of deterioration or of failure to provide suitable support.

Other problems encountered when potatoes were grown in solution cultures relate to the propagation of the plants. It is essential, of course, that the seed potatoes used should be free from diseases and also capable of producing vigorous sprouts. The seed potatoes used in the present work were Maine-grown Green Mountain stock free from viruses except the X virus present to some extent in nearly all seed potatoes. The following procedure was used to grow the potato plants and transfer them to the nutrient solutions. Seed pieces of uniform size (about 25 gm.) were cut and then planted in washed building sand in which they were allowed to remain for about 30 days or until the sprouts were 3 to 3½ inches long and the roots 6 to 8 inches long. Whenever necessary the rest period was broken by treatment with potassium thiocyanate. When the plants had reached the proper size, they were carefully removed from the sand and washed in a stream of tap water. One plant was placed on each nylon-screen support with its roots passing through the ⅛-inch mesh and its stem and small leaves protruding from an opening provided in the upper lid of the wooden support. When transfer was completed, the support and plant were immediately placed on the nutrient solution. Cotton was then packed around the stem to exclude light from the lower compartment of the support and to prevent the subsequent development of vegetative tuber stolons. Normally about 5 days elapsed before the plants recovered from the effects of transfer to the solutions and growth was resumed.

Whether the potato seed piece should be left attached to the young plant or should be removed after the reestablishment of growth is a question that had special significance in the present investigations. If the seed piece was left attached, the plant naturally could obtain from this source a small amount of phosphorus (approximately 25 mg. in the seed piece before sprouting); when the seed piece was

[3] The nylon screen was supplied by E. I. du Pont de Nemours & Co., Wilmington, Del.

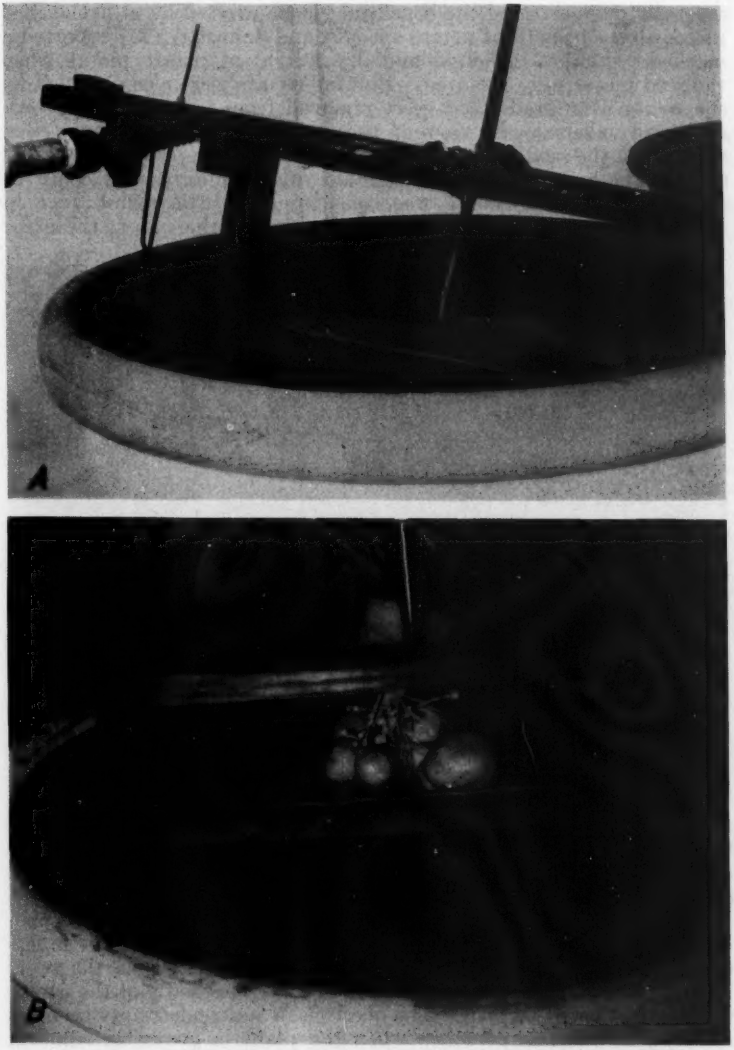

FIGURE 1.—Apparatus designed for growing potatoes in solution cultures: *A*, Plant and seed piece resting on a nylon screen at time of transfer to the solution culture; *B*, plant at time of harvest, showing tuber formation and root development.

removed, however, the growth of the plant was stunted and consequently the yield of the tubers was reduced. Appleman (*1*) experimentally demonstrated the beneficial effects exerted by attached seed pieces on the growth of potato sprouts, and Johnston (*11*) reported a marked reduction in height and dry weight of potato plants when the seed pieces were removed. Similar results were also obtained by the writer after seed pieces were removed from potato plants grown in gravel cultures. In view of these results and the uniform seed stock used, the seed pieces were left attached in the present investigations. The exceedingly small amount of phosphorus that could be supplied from this source was considered of little significance in comparison with the total amount required by the plant; the error

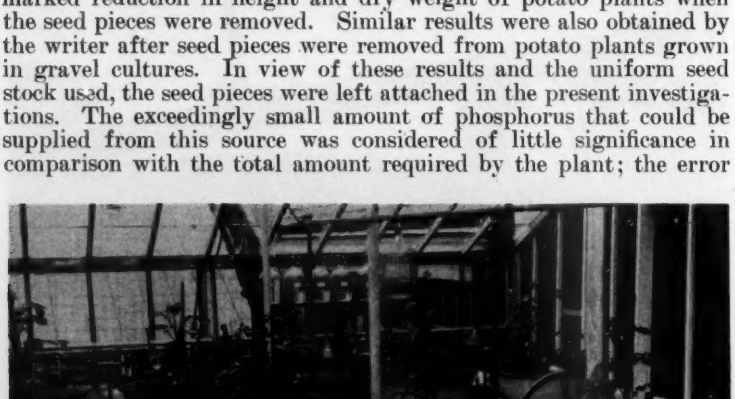

FIGURE 2.—General view of solution-culture apparatus in operation in greenhouse, showing system of aeration, earthenware containers, and float gages.

thus introduced undoubtedly was greatly outweighed by the benefits derived from the favorable growth-promoting effects of the attached seed pieces.

The potato seed piece normally contains enough nutrients to produce sprouts if kept under proper conditions for growth, but unless the young plant obtains additional nutrients by the time the roots have formed its terminal growth will soon be checked and the subsequent growth will be poor. The question of nutrient supply becomes one of particular importance when the plant is grown in solution cultures, especially when the effects of volume and concentration are considered. Johnston and Hoagland (*12*) called attention to the importance of the concentration and supply of nutrients in culture solutions and pointed out their similarity to the intensity and capacity factors of hydraulics. Theoretically, under ideal conditions the nutrient solution should provide the plant with a constantly uniform supply and concentration of the element being studied and in addition should

supply a sufficiency of all other elements required Actually, however, it is very difficult to meet these ideal conditions. The continuous-flow culture may approach this ideal, but even with this method the degree of uniformity attained depends upon the interval between renewals of solution. The continuous-flow method also requires extensive equipment which was not available for the present study; so an alternative method of control was used in which large volumes of solution for the individual cultures were employed. These culture solutions consisted of approximately 95 liters of solution per plant in large vitrified earthenware crocks fitted with the plant supports described on page 2. Each culture was provided with a calibrated float gage so that evaporation and transpiration losses could be replaced daily, thereby maintaining an almost constant volume of solution with the surface one-half inch below the nylon-screen support. Each culture was also aerated continuously by a gentle flow of air sufficient to keep the solution constantly agitated.

The nutrient solutions were made up with tap water and the chemically pure salts listed in table 1. These salts, except for those of iron and the minor elements, were added from 2-molar stock solutions in amounts sufficient to provide the calculated concentration of each element indicated. Except for the minor elements and for phosphate, which was varied, the calculated composition of the solutions was similar to that of solution I recommended by Shive and Robbins (*20*). At the time the nutrient solutions were made up, they were adjusted within the reaction range pH 4.0 to 5.0 with 0.1 N sulfuric acid; thereafter they were readjusted at intervals throughout the course of the experiments. The range pH 4.0 to 5.0 was selected because the results of a series of tests made during the growing season on a number of soil samples from potato fields in the Atlantic Coastal Plain showed a prevailing reaction below pH 5.0.

TABLE 1.—*Calculated composition of nutrient solutions before addition of the phosphate*

Salt (c. p.)	Nutrient	Solution A		Solution B	
		P. p. m.	Milli-equivalents per liter of solution	P. p. m.	Milli-equivalents per liter of solution
Calcium nitrate	Ca	180.32	9.00	180.32	9.00
	NO_3	558.07	9.00	558.07	9.00
Potassium chloride	K	89.93	2.30	89.93	2.30
	Cl	81.56	2.30	81.56	2.30
Magnesium sulfate	Mg	55.94	4.60	55.94	4.60
	SO_4	220.94	4.60	220.94	4.60
Ammonium sulfate	NH_3	23.83	1.40	11.91	.70
	SO_4	67.24	1.40	33.62	.70
Boric acid	B	.189	.0515	.094	.0258
Manganous sulfate	Mn	.262	.0095	.065	.0024
	SO_4	.458	.0095	.115	.0024
Copper sulfate	Cu	.272	.0086	----------	----------
	SO_4	.411	.0086	----------	----------
Zinc sulfate	Zn	.242	.0074	----------	----------
	SO_4	.356	.0074	----------	----------
Ferric citrate	Fe	3.0	.1612	3.0	.1612

It was intended that an excess of all nutrients other than phosphate should be maintained in the nutrient solutions, and occasional colorimetric determinations made on solution samples showed that this

was accomplished. The levels of phosphate in the solutions, however, were maintained by making phosphorus determinations on filtered samples at least once each weekday and adding PO_4 as required from a solution of monopotassium phosphate. It was not possible to make any tests or additions of PO_4 on Sundays. Phosphorus was determined with a photoelectric colorimeter by the Denigès method as outlined by Truog (*25*).

Ferric citrate was added to the fresh nutrient solutions at the rate of 3 p. p. m. of Fe, and thereafter 1 p. p. m. of Fe was added at weekly intervals or as needed. No evidence of iron chlorosis appeared at any time while this procedure was being followed, but the iron additions caused a gradual precipitation of ferric phosphate, which accumulated in the bottom of the tanks. Although this precipitate was insoluble, an attempt was made to avoid its formation by supplying iron to the plants in separate solutions. To evaluate this procedure there was run a special test in which plants were grown in the large tanks without iron but were allowed to remain overnight several times a week in separate solutions containing 5 or 10 p. p. m. of Fe. After each treatment the plant roots were thoroughly washed in tap water before being replaced in the regular solutions. Within 10 days the growth of these plants was markedly affected; the leaves had developed a dull, light-green color and subsequently the terminal growth stopped After 50 days only very small tubers had formed. It was concluded that this method of supplying iron, although desirable for the reasons stated, was unsatisfactory for growth of the potato plant. Although direct addition of ferric citrate to the nutrient solution was objectionable because of the gradual precipitation of ferric phosphate, the growth response of the plants by this procedure was satisfactory and there were no signs of iron deficiency. Under these conditions, however, the disappearance of phosphate from the nutrient solutions could not be taken as a measure of its rate of absorption by the plants. Therefore, an attempt was made by frequent renewals of phosphate and by use of large volumes of solution per plant to maintain a fairly constant control of the phosphate concentration of the solution in contact with the plant roots and to indicate phosphorus absorption by chemical analysis of the plants.

EXPERIMENTAL RESULTS

EXPERIMENT 1

The first experiment was conducted with nutrient solution A (table 1) with five levels of PO_4—6.0, 3.0, 1.5, 0.6, and 0.3 p. p. m. Each nutrient culture with one potato plant was replicated three times at each phosphate level. The Green Mountain potato plants were transferred to the solutions on December 8, 1943, and harvested on February 8, 1944.

Growth Data

Growth of potato tops, although not necessarily a reliable index of tuber yield, may be used nevertheless as an indication of nutrient response. Accordingly, the rate of top growth was determined by measuring the height of the plants each day. The results of these measurements are plotted at 10-day intervals in figure 3, *A*. It will be noted that after 40 days the plants at the levels of 0.3 and 0.6 p. p. m.

of PO_4 showed definite reductions and finally cessation of growth, whereas the plants at higher PO_4 levels continued to grow. The failure of the plants in general to make quantitative growth responses according to the amounts of PO_4 in the solutions is no doubt due to inherent irregularities in the top growth of individual plants, which occur normally in the potato. Because of this fact generalizations cannot be attempted with a small number of plants, but from the rates of growth obtained after the plants had been on the solution 40 days

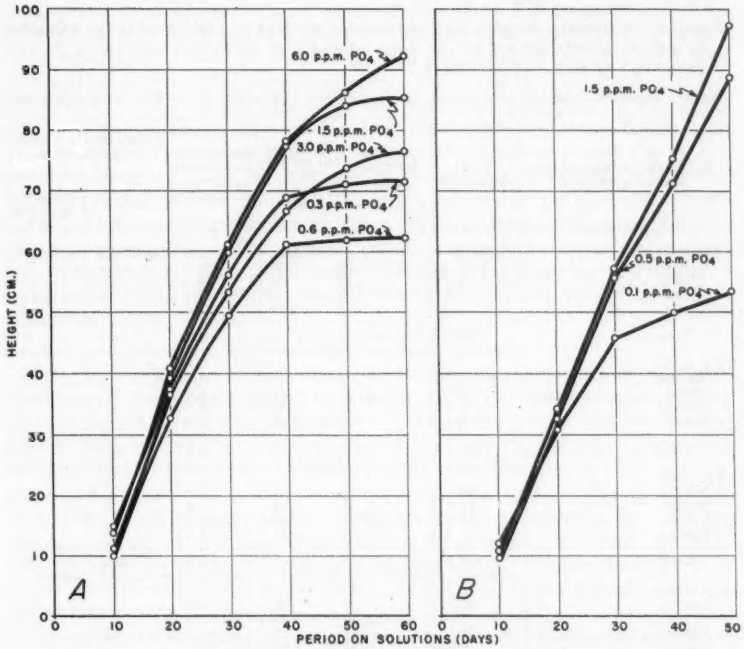

FIGURE 3.—Curves showing relation between average height and number of days on solution of potato plants grown on nutrient solutions containing different concentrations of phosphate: *A*, Experiment 1; *B*, experiment 2.

it would appear that under the conditions of the experiment a PO_4 concentration greater than 0.6 p. p. m. was required for optimum top growth.

CHEMICAL DATA

At the end of a 62-day period the plants were harvested and separate dry weights were obtained for the leaves, stems, tubers, and roots dried at 55° C. for 48 hours. The dried material was then ground in a Wiley mill to pass a 40-mesh sieve. Samples of the dried material were ashed, and phosphorus was determined by the method of Fiske and Subbarow (*7*). The resulting data are presented in table 2.

The data in table 2 show clearly that a definite reduction in the amount of dry matter occurred when only 0.6 or 0.3 p. p. m. of PO_4

was supplied in the nutrient solution. When the PO_4 content of the solution was raised to 1.5 p. p. m., more dry matter was produced; but further increases up to 6.0 p. p. m. had little effect. It is of interest to note (table 2) that the two lower PO_4 levels produced increases in the root-top ratios. This is in agreement with the results obtained by Sommer (*22*) with tomatoes, cotton, and wheat. It is apparent in the present case, however, that the increases in these ratios were caused by marked reductions in top growth and not by increases in root growth, as found by Sommer.

TABLE 2.—*Total dry weights and phosphorus content of and phosphorus absorbed by potato plants grown for 62 days in nutrient solutions containing various quantities of PO_4, experiment 1*

[Values based on 3 plants each grown in an individual solution at the PO_4 level indicated]

PO_4 content of nutrient solution and plant part	Total dry weight	Root-top ratio	Phosphorus content (dry-weight basis)	Amount of phosphorus absorbed by plants	Phosphorus in nutrient solutions	
					Added	Recovered in plants
6.0 p. p. m.:	*Grams*		*Percent*	*Millimols*	*Millimols*	*Percent*
Leaves	57.5		0.73	13.5		
Stems	16.3		.66	3.5		
Tubers	117.0	0.118	.56	21.1		
Roots	8.7		2.25	6.3		
Total	199.5			44.4	78.0	56.9
3.0 p. p. m.:						
Leaves	45.4		.79	11.6		
Stems	11.5		.55	2.0		
Tubers	107.4	.107	.46	15.9		
Roots	6.1		2.47	4.9		
Total	170.4			34.4	58.4	58.9
1.5 p. p. m.:						
Leaves	49.1		.62	9.8		
Stems	13.0		.43	1.8		
Tubers	118.8	.118	.48	18.4		
Roots	7.3		2.33	5.5		
Total	188.2			35.5	48.4	73.3
0.6 p. p. m.:						
Leaves	33.7		.31	3.4		
Stems	7.7		.20	.5		
Tubers	93.7	.126	.33	10.0		
Roots	5.2		1.92	3.2		
Total	140.3			17.1	27.5	62.2
0.3 p. p. m.:						
Leaves	31.2		.30	3.0		
Stems	8.4		.12	.3		
Tubers	90.8	.139	.28	8.2		
Roots	5.5		1.19	2.1		
Total	135.9			13.6	18.4	73.9

The percentage of phosphorus in the dry matter was also markedly affected by the level of PO_4 maintained in the nutrient solution. As the PO_4 content of the nutrient solutions was decreased from 6.0 to 0.3 p. p. m., the percentage of phosphorus in the dry matter also decreased, but not in proportion to the amount of PO_4 supplied. Similar results were reported by Lyness (*14*) in a study of the phosphorus absorption by corn growing in sand cultures

The sharp reductions in the percentages of phosphorus, especially in the leaves and stems, at the 0.6 p. p. m. level, the further reductions

obtained in the stems and tubers at the 0.3 p. p. m. level, and the fact that growth in each case practically stopped at 40 days, all strongly suggest an inadequate supply of PO_4 at these levels. Although specific phosphorus-deficiency symptoms did not appear in the plants growing at the two lower PO_4 levels, their general appearance was definitely subnormal and their dry weights were less than those obtained at the higher phosphate levels.

The data in table 2 showing the amount of phosphorus absorbed by the plants clearly indicate that marked reductions in absorption took place in the solutions containing less than 1.5 p. p. m. of PO_4. A total of 35.5 millimols of phosphorus was absorbed from the solution containing 1.5 p. p. m. of PO_4, but the totals were only 17.1 and 13.6 millimols from the solutions with PO_4 levels of 0.6 and 0.3 p. p. m., respectively. Although the total millimols of phosphorus absorbed by the plants differed at each level of phosphate supplied, the tubers invariably contained the largest amounts of phosphorus and the leaves the next largest. The percentages of the added phosphate recovered by the plants were in general fairly high, but the percentages of recovery were considerably lower from the solutions containing 3.0 and 6.0 p. p. m. of PO_4. The lowest recovery was 56.9 percent; therefore the proportion of phosphate added to the solutions which remained unaccounted for by plant removal probably did not exceed about 43 percent.

EXPERIMENT 2

The second experiment was conducted primarily to study the reaction of the potato plant to a level of PO_4 lower than that used in experiment 1; only three levels (1.5, 0.5, and 0.1 p. p. m.) were used. The number of replications was increased from three to five, but each culture, as in experiment 1, consisted of only one plant. In this experiment nutrient solution B (table 1) was used; this differed slightly from solution A, principally in the omission of copper and zinc. Other changes made in solution B include a 50-percent reduction in NH_3 nitrogen and reductions in manganese and boron. These changes were made after preliminary tests, in which solution B produced considerably better growth than solution A, had been run.

Green Mountain potato plants were transferred to the solutions on February 8, 1945, and thereafter the experimental procedure followed was the same as that outlined on page 5.

Growth Data

The plants in experiment 2 showed a marked improvement over those in experiment 1 as they were more nearly like field-grown plants. The rate of top growth for each PO_4 level is shown graphically in figure 3, *B*, in which the height measurements (averages of three plants) were plotted for 10-day intervals. Comparison of these growth curves shows that within a period of 20 to 25 days the solution supplying the lowest level of PO_4, 0.1 p. p. m., had a definite retarding effect on top growth. After 30 days the average height was only 46 cm., and thereafter the plants made little terminal growth; their upper leaves were small and dark green and the margins were noticeably wrinkled (fig. 4, *B*). At the two higher PO_4 levels (1.5 and 0.5 p. p. m.) growth was good up to 50 days; the average plant heights were

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89 and 98 cm., respectively, and the leaves were normal in appearance. Although there was little difference in the height of the plants and their rate of elongation in these two solutions, the plants in the 1.5 p. p. m. solutions had thicker stems and produced considerably more secondary growth. The relative growth responses of tops and roots to the three levels of PO_4 are illustrated in figures 5 and 6.

FIGURE 4.—Potato plants showing effect of phosphate on terminal leaf growth: *A*, Nutrient solution contained 1.5 p. p. m. of PO_4; *B*, nutrient solution contained 0.1 p. p. m. The lower leaves in *B* were dull and yellowish green, whereas the terminal ones were dark green with wrinkled margins.

FIGURE 5.—Potato plants 32 days after transfer to the solution cultures, showing effect of different phosphate levels on the top growth. Note the secondary growth at 1.5 p. p. m. of PO_4.

CHEMICAL DATA

After experiment 2 had been running for 30 days, it became increasingly difficult at certain periods to maintain the phosphate levels in the solutions supplying 0.1 p. p. m. of PO_4. This was particularly evident on bright days, when several additions of PO_4 were needed to bring the solutions up to the required level. Apparently the plants in these solutions had reached a stage of growth during which their phosphate requirements could only be satisfied by a virtually continuous supply at the 0.1 p. p. m. level.

The problem of maintaining levels of PO_4 in solution cultures, especially when they approach a critical point for plant growth,

FIGURE 6.—Roots of potato 51 days after plants were transferred to solution cultures, showing effect of different phosphate levels on growth. The darker color at 0.5 p. p. m. and 0.1 p. p. m. of PO_4 is due to a coating of ferric iron.

obviously is inseparably connected with the absorption of the plants growing thereon. Under active growing conditions none of the levels used in the present experiments can be regarded as constantly maintained, since the amount of PO_4 in the solutions under such conditions can change very rapidly. According to Brooks (*4*) equilibrium cannot be attained while cell growth is actively taking place; during that time ions move into the plant rapidly, and it is now generally understood that some ions move out also, but at a slower rate (*8*).

Since the amounts of PO_4 found in the nutrient solutions were recorded each day, these records constitute a means of appraising the PO_4 status of the nutrient solutions during the course of the experiment. As it is not possible to present these data for all 15 tanks,

typical data were selected for 2 tanks at each PO_4 level and these were plotted in figure 7. Such values represent the concentration of PO_4 found in the solutions each morning; therefore, it is reasonable to assume, except where a surplus still existed, that the average PO_4 concentration in contact with the plant roots had been greater during the preceding 24 hours. Whenever the chemical analysis showed an amount of PO_4 below the theoretical value for a particular nutrient

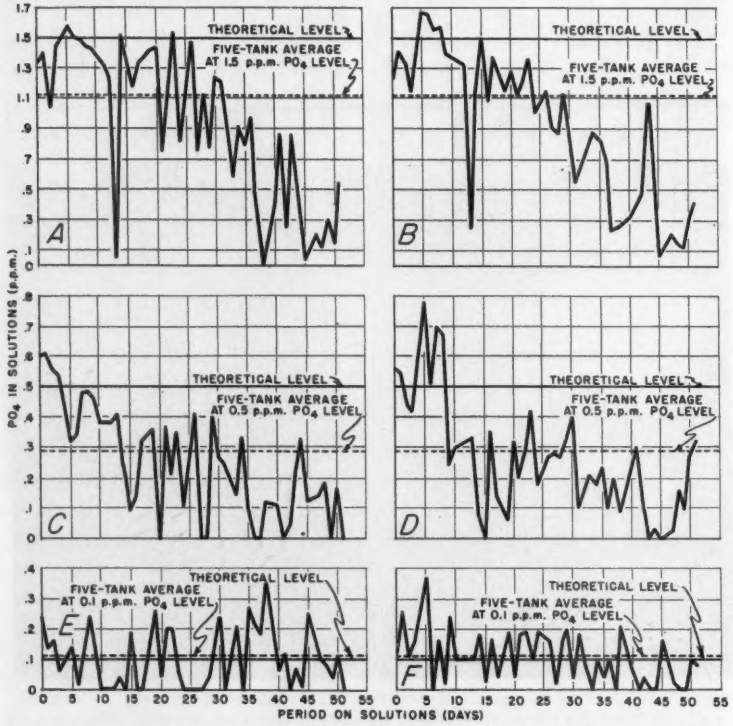

FIGURE 7.—Curves showing phosphate found each morning in two typical tanks at each PO_4 level, experiment 2: *A* and *B*, 1.5 p. p. m.; *C* and *D*, 0.5 p. p. m.; *E* and *F*, 0.1 p. p. m. Five-tank averages: 1.5 p. p. m., 1.124 ± 0.026; 0.5 p. p. m., 0.273 ± 0.013; 0.1 p. p. m., 0.116 ± 0.009.

solution, sufficient monopotassium phosphate was added to raise it to the proper level; otherwise no phosphate additions were made. The fluctuations in PO_4 content of the nutrient solutions illustrated in figure 7 obviously were not caused entirely by plant absorption, since the greatest recovery by the plants was not above 85.5 percent. (See table 4.) Chemical reactions in the nutrient solutions and possibly to a less extent biological activity are suggested as contributing factors, but it seems reasonable to attribute the greater part of these fluctuations to plant absorption. In this connection it is important to recognize that, because of the large volume of solution provided

per plant and the frequency of PO_4 fortification, any critical reductions in PO_4 content that may have occurred could have been of only short duration.

Because of the difficulty experienced in maintaining the PO_4 level of the 0.1 p. p. m. solutions during certain periods, as previously mentioned, a decision was made to harvest two replications at each PO_4 level after they had been on the solutions 33 days and to allow three replications to remain until tubers could form. Accordingly, plants selected at random were harvested on March 13, 1945, dried, ground, and analyzed for phosphorus as previously described. At the time of harvest the leaves of these plants were divided into three portions according to their vertical position on the plant: upper third, middle third, and lower third. These samples were analyzed separately in an attempt to determine more fully the relative distribution of phosphorus within the plant at an early stage of development. The data obtained are presented in table 3.

TABLE 3.—*Total dry weights and phosphorus content of and phosphorus absorbed by potato plants grown for 33 days in nutrient solutions containing various quantities of PO_4, experiment 2*

[Each plant grown in an individual solution at the PO_4 level indicated]

Nutrient solution containing indicated PO_4	Phosphorus in duplicate potato plants					Total dry weight	Amount of phosphorus absorbed by plants [1]	Phosphorus in nutrient solutions	
	Upper leaves	Middle leaves	Lower leaves	Stems	Roots			Added	Recovered in plants
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Millimols*	*Millimols*	*Percent*
1.5 p. p. m.	0.97	0.71	0.59	0.56	1.32	31.8	7.49	18.17	41.2
	.88	.68	.60	.56	1.22				
0.5 p. p. m.	.71	.45	.34	.34	.74	29.3	4.54	11.17	40.6
	.64	.40	.33	.28	.69				
0.1 p. p. m.	.38	.20	.18	.12	.55	18.2	1.55	2.75	56.4
	.34	.22	.21	.10	.39				

[1] Obtained from percentage of PO_4 and dry weight of each plant part.

The effects of phosphate supply on the percentage of phosphorus in the plants after 33 days are brought out by the data in table 3. It is clear that when the concentration of phosphate supplied was below 1.5 p. p. m. the percentage of phosphorus in the different plant parts was definitely reduced in every case and the total amount of phosphorus absorbed was much lower. It will be noted, however, that although the amount of phosphorus absorbed from the 0.5 p. p. m. solution was considerably less than that absorbed from the 1.5 p. p. m. solution, the corresponding amounts of dry matter and heights of plant (fig. 3, *B*) were similar. Evidently these results illustrate the general concept stated by Hoagland and Davis (*9, p. 108*) that "two solutions may produce plants of equal dry weight, but of very different composition."

As might be expected, the results of the analyses given in table 3 show that the upper leaves contained the highest percentages of phosphorus. This was true at all three levels of phosphate supplied. The lower leaves, on the other hand, contained the lowest percentages of phosphorus; the middle leaves may be regarded as intermediate in phosphorus content. These results suggest that mature leaves from

the middle of the plant may be taken as representative when potato foliage is sampled to determine the general phosphorus level in the plant.

The three remaining replications of experiment 2 were harvested April 2, 1945, after being on the nutrient solutions 53 days. Data pertaining to these more mature plants are given in table 4. The relation between the quantities of PO_4 supplied and the total dry matter produced (table 4) was more pronounced than was the case with the earlier harvested plants (table 3). After the plants had remained on the 1.5 and 0.5 p. p. m. PO_4 solutions for 53 days, there was a marked difference in their dry-matter content, which was not obtained at 33 days. In general, all parts of the more mature plants except the tubers at 0.5 p. p. m. of PO_4 were found to have a lower dry-matter content when the phosphate supply was reduced.

TABLE 4.—*Total dry weights and phosphorus content of and phosphorus absorbed by potato plants grown for 53 days in nutrient solutions containing various quantities of PO_4, experiment 2*

[Values based on 3 plants each grown in an individual solution at the PO_4 level indicated]

PO_4 content of nutrient solution and plant part	Total dry weight	Root-top ratio	Phosphorus content (dry-weight basis)	Amount of phosphorus absorbed by plants	Phosphorus in nutrient solutions	
					Added	Recovered in plants
1.5 p. p. m.:	*Grams*		*Percent*	*Millimols*	*Millimols*	*Percent*
Leaves	202.2	0.128	0.59	38.5		
Stems	78.5		.45	11.4		
Tubers	50.2		.54	8.8		
Roots	36.0		.80	9.3		
Total	366.9			68.0	87.0	78.2
0.5 p. p. m.:						
Leaves	113.0	.156	.34	12.4		
Stems	38.1		.24	3.0		
Tubers	75.6		.38	9.3		
Roots	23.5		.48	3.6		
Total	250.2			28.3	40.8	69.4
0.1 p. p. m.:						
Leaves	39.5	.220	.17	2.2		
Stems	10.4		.16	.5		
Tubers	39.9		.21	2.7		
Roots	11.0		.32	1.1		
Total	100.8			6.5	7.6	85.5

The percentage of phosphorus in the dry matter of the plants (table 4) was also affected when the supply of phosphate was reduced below 1.5 p. p. m. Although the percentages of phosphorus in all parts of the plant at the 0.5 p. p. m. level, and the dry matter in most, were definitely reduced, these indications of phosphorus insufficiency appear to have had little effect on the height of the plants (fig. 3, *B*) throughout the experiment. At the 0.1 p. p. m. level, however, the reductions in both percentage of phosphorus and amount of dry matter were pronounced, growth was markedly affected, and phosphorus-deficiency symptoms developed in the plants. Three stages of absorption based on the percentage content of a given nutrient in the plant were recognized by Macy (*15*). He suggested the following criteria to describe

them: A "critical percentage" stage above which there is evidence of "luxury consumption," a "poverty adjustment" stage in which plant growth is adjusted to a limited nutrient supply, and finally a "minimum percentage" stage in which growth practically stops. In the present experiments a "critical percentage" stage apparently developed in the potato plants at the 1.5 p. p. m. PO_4 level, since some evidence of "luxury consumption" appeared above this level (table 2). A "poverty adjustment" stage evidently was reached near the 0.5 p. p. m. level and a "minimum percentage" stage at about the 0.1 p. p. m. level.

Compared with the total amount of phosphorus absorbed from the 1.5 p. p. m. PO_4 solution, that absorbed from the 0.5 p. p. m. solution was reduced more than 50 percent and that from the 0.1 p. p. m. solution 90 percent. The highest percentage recovery by the plants, however, occurred at the lowest phosphate level, as in the case of the earlier harvested plants. In general, when the concentration of phosphate in the nutrient solutions was decreased, the amount of phosphorus found in all plant parts was lower, but these reductions were greater in the stems and leaves.

DISCUSSION

Although there were periods in the growth of the potato plants when the demand for phosphate increased, these appeared to be due more to environmental conditions that were favorable for growth than to any particular development of the plant. For instance, there was no indication of a sustained increase in the demand for phosphate when tuber growth commenced or at the initiation of the blossom primordia. The extensive root development obtained in the solution cultures early in the growth of the plant and the favorable conditions existing for absorption of phosphate may account for the failure to detect any pronounced increase in demand for phosphate at the periods mentioned. Under conditions of growth in the soil, however, supplying phosphate early seems desirable. The work of Carolus (*6*) indicated that a peak in the absorption of phosphate by potatoes occurred 50 to 80 days after planting. It is not known, however, whether the demand for phosphate can become critical at any stage during this period of growth. This point would seem to warrant further study, since it has an important bearing on the question of the proper time to supply readily available phosphate for potatoes as well as the amount to be used in relation to the less readily available phosphate reserves in the soil. The time phosphate is supplied as well as the amount supplied may be important in enabling the plant not only to produce early, vigorous growth but also to accumulate a phosphorus reserve for its subsequent requirements when less phosphate is usually available.

It should be obvious that the results obtained in the present investigations by solution-culture procedure cannot serve directly as a basis for recommendations in the field and that data obtained on a small number of potato plants cannot provide a sound basis for generalizations. However, these data indicate very definitely that the percentage of phosphorus in the potato plants and the production of dry matter were both markedly affected by the levels of phosphate supplied in the

nutrient solutions. There was also a general relation between the amount of phosphorus absorbed by the plants and the level of phosphate supplied; but, as might be expected, this relation was not proportional. Although the plants in the solutions maintained at about 0.1 p. p. m. of PO_4 were unable to obtain sufficient phosphorus for their growth requirements, there was no evidence to indicate that this failure was due to their inability to absorb phosphorus from such dilute solutions. Under the conditions of the experiments the potato plants probably developed phosphorus-deficiency symptoms in these dilute solutions when the rate of absorption proved inadequate for the needs of the plant. However, if a potato variety other than Green Mountain had been used or the experimental procedures had been modified, it is entirely possible that the results obtained in the most dilute solutions might have been somewhat altered.

SUMMARY

Experiments were conducted with a specially devised nutrient-solution apparatus to determine the minimum phosphate requirement of the potato plant (Green Mountain variety). Low levels of phosphate approximating those that have been found in the soil solution were used in the nutrient solutions, and the solutions were analyzed daily for phosphate and fortified as required by adding monopotassium phosphate. Approximately 95 liters of nutrient solution was supplied each plant. Support for the tubers and the plant above the surface of the solution was provided by a ⅛-inch mesh nylon screen.

Under the conditions of the experiments the potato plants made excellent growth in the nutrient solutions maintained at a phosphate concentration of approximately 1.5 p. p. m. When the phosphate concentration was reduced to 0.5 p. p. m., the height of the plants was only slightly reduced but the production of dry matter and the percentage of phosphorus in the plants were definitely lowered. When the phosphate concentration of the solutions was reduced to 0.1 p. p. m., however, the plants were much smaller, phosphorus-deficiency symptoms developed, and there was a pronounced reduction in both dry matter and percentage of phosphorus. The results obtained at the phosphate concentrations of 1.5, 0.5, and 0.1 p. p. m. may be identified, respectively, with the three stages of nutrient absorption suggested by Macy (*15*): namely, "critical percentage," "poverty adjustment," and "minimum percentage."

The results of phosphorus determinations made on the upper, middle, and lower leaves of the plants suggest that a sample of mature leaves selected from the middle of potato plants can be used for analysis to indicate the general phosphorus uptake.

In general, the amount of phosphorus absorbed increased as the concentration of phosphate in the solutions increased, but the percentage of phosphorus recovered by the plants was greater in the most dilute solutions.

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REACTIONS OF CABBAGE VARIETIES TO MOSAIC VIRUSES [1]

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INTRODUCTION

In recent years considerable attention has been given to the description and identification of viruses affecting cabbage (*Brassica oleracea* var. *capitata* L.) and related plants (*1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13*).[2] In 1942 a survey was made of the relative resistance of a number of selfed and hybrid lines of Wisconsin All Seasons cabbage to mosaic. Some promising selections were found; incorporation of their resistance to mosaic with resistance to yellows and desirable horticultural type is under way (*12*). During the search for possible breeding material it was thought wise to test the more common commercial varieties in order to learn in which varieties resistance is most needed and in which it might be found. The work was carried out in the seed-growing district of the Puget Sound section in western Washington.

MATERIALS AND METHODS

Practically all cabbage varieties of commercial importance are grown as seed crops in the Puget Sound section. The seedlings of the various varieties are usually grown together in large plant beds, and later they are transplanted to fields where they are left for seed production. Under the cultural methods used prior to 1945, 60 to 75 percent of the plants became infected with mosaic while in the plant beds (*3*). Because seedlings were so widely infected, some data were taken in farmers' seed fields. Additional data came from experimental field plots.

Varieties in farmers' fields for the 1943–44 seed crop were rated by a disease-index system. As plants of all varieties had a common exposure while in the plant beds, rating individual diseased plants in transplanted fields according to severity of symptoms was used to obtain a measure of the relative susceptibility of the various varieties.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 30.

It was necessary to rate a large number of plants in order to minimize variations between locations. One set of ratings was made in the fall 30 to 50 days after transplanting, and another was made in the same fields the following summer about 6 weeks prior to seed harvest. Notes were taken on infected plants only, and all in the row that was being checked were rated. Sample rows were selected from different areas in the field. Each plant was placed in one of four classes arbitrarily chosen according to the severity of symptoms described in table 1. In calculating the disease index, the number of plants in each class was multiplied by the class value, the sum of the products was multiplied by 100, and the result was divided by 4 times the total number of plants. Thus if all the plants were in class 1 the index would be 25; if all were in class 4, the most severe class, the index would be 100.

In 1944 several varieties were grown for head cabbage in randomized, replicated plots. These were transplanted into paired rows, and 2 weeks later the plants in one row of each pair were inoculated mechanically with virus strains (p. 21). The other row of each pair served as a check. Because of severe infection of check plants by aphid activity no yield data were taken; the plants were rated, however, by a disease-index system identical with that just described.

TABLE 1.—*Description of disease-severity classes of infected cabbage plants*

Severity class	Class value	Severity of symptoms	Class description for—	
			Head cabbage	Seed cabbage
1	25	Mild	Mild mottling and vein clearing; or either of these by itself; no necrosis; no conspicuous stunting.	Mild mottling, stunting, and necrosis of leaves; or mild mottling and stunting of leaves; or mild necrosis of leaves; no necrosis of stalk, pods, or pedicels.
2	50	Moderate	Relatively severe mottling, distortion, and stunting; or necrosis with little or no mottling.	Marked chlorosis and necrosis of leaves; or either of these by itself; defoliation not greater than 25 percent; very little necrosis of stalk or pods.
3	75	Severe	Severe mottling with leaf distortion and moderate necrosis; marked stunting.	Severe chlorosis and necrosis of leaves, resulting in defoliation up to 50 percent; moderate necrosis of stalk, pedicels, and pods.
4	100	Very severe	Severe mottling, leaf distortion, stunting, necrosis, and defoliation.	Severe chlorosis and necrosis of leaves, resulting in defoliation up to 100 percent; severe necrosis of stalk, pedicels, and pods.

In 1945 a similar plot was used, but the seedlings were grown in greenhouse flats and inoculated before being transplanted. In this experiment a slightly different system of rating disease severity was used. Each of 25 plants in each replicate was rated and placed in 1 of 4 severity classes for each of 3 different symptom categories as shown in table 2. The disease index was calculated by multiplying the total number of plants in each class by the class value and then dividing the sum of these products (total score) by 3 (since there were 3 symptom categories) times the number of plants being rated (25 in the example). This method proved to be much more effective in rating disease severity than the one in which all symptoms were combined into 1 category of severity. It is realized that there can be no accurate quantitative measurement of severity of disease symptoms,

TABLE 2.—*Method of determining disease index of 25 cabbage plants in experimental plots, 1945*

Severity class	Class value	Severity of symptoms	Plants in indicated symptom category			Total for 3 categories	Score [1]
			Stunting-leaf distortion	Mottling-chlorosis	Necrosis		
			Number	*Number*	*Number*	*Number*	
1	25	Mild	8	7	7	22	550
2	50	Moderate	10	10	12	32	1,600
3	75	Severe	3	6	6	15	1,125
4	100	Very severe	4	2	0	6	600
Total			25	25	25	75	[2] 3,875

[1] Obtained by multiplying the total number of plants in each class by the class value.
[2] Disease-index value=51.7 (determined by dividing the total score by 3 times the number of plants measured, as each plant was classified in 3 categories).

but it is felt that this method gave a fairly true picture of the relative varietal susceptibility.

In the Puget Sound section, as in the Midwest (*13*), cabbage mosaic is caused by two unrelated viruses (virus A, a strain of turnip virus 1 Hoggan and Johnson, and virus B, a strain of cauliflower virus 1 Tompkins) which normally occur together in plants. The data on plants from the 1943–44 farmers' fields represent, therefore, the disease as it occurs naturally in this section. In the 1944 plot, plants were inoculated with a mixture of isolates of viruses A and B obtained from the University of Wisconsin. In the 1945 plot the varieties were inoculated with a mixture of Wisconsin viruses A and B, with a mixture of viruses A and B occurring in the Puget Sound section, and with a mixture of Wisconsin virus B and the California black ring virus (*10*) (another strain of turnip virus 1 originally obtained from the University of California). Extensive greenhouse tests have shown that the virus strains producing mosaic in the Puget Sound section are identical with those occurring in the Midwest (*13*) and are not the strains reported as prevalent in California (*6*, *10*).

The varieties [3] studied were of the following types:

Flatheaded:
- All Head Early
- All Head Select
- Premium Late Flat Dutch
- Stein's Early Flat Dutch
- Succession
- Wisconsin All Seasons

Ballhead-Hollander:
- Bugner
- Danish Ballhead
- Ferry's Hollander
- Improved Wisconsin Ballhead
- Penn State Ballhead
- Wisconsin Ballhead
- Wisconsin Hollander

Pointedheaded:
- Charleston Wakefield
- Early Jersey Wakefield
- Jersey Queen

Roundheaded:
- Copenhagen Market
- Early Round Dutch
- Globe
- Glory of Enkhuizen
- Golden Acre
- Marion Market
- Midseason Market
- Resistant Detroit

[3] Yellows-resistant varieties were from stocks of the Wisconsin Cabbage Seed Co. All other varieties were from stocks of Ferry-Morse Seed Co. except those in farmers' fields in which Stein's Early Flat Dutch, Charleston Wakefield, and Early Round Dutch from stocks of the Associated Seed Growers, Inc., were used.

VARIETAL REACTIONS

SEED FIELDS IN 1943–44

SYMPTOM SEVERITY

In tables 3 and 4 are given the varietal reactions of cabbage 30 to 50 days after transplanting to farmers' fields and just before seed harvest, when the disease had incubated for 10 or 11 months.

A study of table 3 will reveal that the varieties represented a distinct gradient of susceptibility but could be divided roughly into three classes. In the least susceptible group were All Head Select, Wisconsin All Seasons, Globe, and Stein's Early Flat Dutch. Except for Globe all of these are of the flatheaded type and are very similar in time of maturity. No counts were made on Succession, another flatheaded variety, but observations indicated that it would fall in this group. The intermediately susceptible group included all the early and midseason roundheaded varieties tested except Globe, in addition to All Head Early, Charleston Wakefield, Early Jersey Wakefield, and Premium Late Flat Dutch. Early Jersey Wakefield was at the top of this group. All roundheaded varieties in this group were remarkably similar in their reaction. The ballheads comprised the most susceptible group; Ferry's Hollander was distinctly the most severely affected variety of this group.

It is apparent that during the seed stage (table 4) the varieties occupied relatively the same position of susceptibility as in the fall. Then too, Wisconsin All Seasons, Globe, Stein's Early Flat Dutch, and All Head Select comprised the least susceptible group. Also, the roundheaded varieties except Globe were intermediate in susceptibility, and the ballheads with the exception of Wisconsin Hollander were the most susceptible. It was surprising to find Wisconsin Hollander showing only moderately severe symptoms in view of its extreme susceptibility under midwestern conditions. All varieties showed a higher disease-index value during the seed stage than during

TABLE 3.—*Relative susceptibility of 18 cabbage varieties to cabbage mosaic 30 to 50 days after transplanting, 1943–44*

Variety	Total plants checked	Plants in severity class				Disease-index value
		1	2	3	4	
	Number	*Number*	*Number*	*Number*	*Number*	
All Head Select	563	506	57	0	0	27.5
Wisconsin All Seasons	734	599	132	3	0	29.7
Globe	683	429	254	0	0	34.3
Stein's Early Flat Dutch	686	396	262	28	0	36.6
Early Jersey Wakefield	693	310	370	13	0	39.3
Premium Late Flat Dutch	738	258	420	60	0	43.3
Glory of Enkhuizen	812	203	585	24	0	44.5
Charleston Wakefield	703	225	404	74	0	44.6
Early Round Dutch	637	146	460	31	0	45.5
Resistant Detroit	704	156	500	48	0	46.2
Golden Acre	629	144	432	53	0	46.4
Marion Market	745	179	485	81	0	46.7
All Head Early	750	124	548	78	0	48.5
Copenhagen Market	674	127	449	98	0	48.9
Wisconsin Hollander	532	44	326	162	0	55.5
Danish Ballhead	712	51	339	305	17	60.1
Penn State Ballhead	696	46	330	300	20	60.6
Ferry's Hollander	674	23	267	363	21	64.2

TABLE 4.—*Relative susceptibility of 15 cabbage varieties to cabbage mosaic during seed stage, 1943–44*

Variety	Total plants checked [1]	Plants in severity class				Disease-index value
		1	2	3	4	
	Number	*Number*	*Number*	*Number*	*Number*	
Wisconsin All Seasons	296	101	152	43	0	45.1
Globe	317	50	220	47	0	49.8
Stein's Early Flat Dutch	316	38	210	50	18	53.8
All Head Select [2]	136	17	85	30	4	53.9
Marion Market	331	30	168	90	43	61.0
Early Jersey Wakefield	322	13	157	145	7	61.3
Wisconsin Hollander	254	8	117	107	22	64.1
Glory of Enkhuizen	394	9	180	175	30	64.3
Resistant Detroit	301	18	117	134	32	65.0
Copenhagen Market	299	25	81	130	63	69.3
Golden Acre	243	20	59	117	47	69.7
Early Round Dutch	329	4	114	151	60	70.3
Charleston Wakefield	370	17	124	135	94	70.7
Penn State Ballhead	344	0	93	140	111	76.3
Danish Ballhead	311	0	49	118	144	82.6

[1] In all varieties the presence of downy mildew made an accurate estimation of virus defoliation difficult, but the effects of the 2 diseases were separated as much as possible.
[2] Severe development of downy mildew confused diagnosis in this variety.

the immature-head stage. This increase tended to be greatest for the least susceptible varieties and least for the most susceptible ones.

SYMPTOM TYPE

As previously mentioned, mosaic is caused by two unrelated viruses which normally occur together (*13*). Virus A characteristically produces chlorotic spotting or chlorotic ringing of leaves, which is later followed by black necrotic rings or irregular necrotic spots. Virus B, the other component, characteristically produces chlorotic vein clearing, vein banding, and general, diffuse, deficiencylike chlorosis. When the two viruses occur together, the disease reaction is more severe than when either virus occurs alone.

In table 5 the relative prominence of various types of symptoms is given for each variety. In the immature-head stage the four most tolerant varieties characteristically showed mild mottling and mild vein clearing and general chlorosis. Virus B symptoms usually predominated over those of virus A. The chlorosis, which resembled that of a mineral deficiency, was different from the chlorosis resulting from severe mottling as shown in roundheaded types. In the intermediate group there was a great diversity of symptoms. Early Jersey Wakefield, Charleston Wakefield, All Head Early, and Premium Late Flat Dutch showed much more pronounced vein clearing and vein banding than other varieties. All roundheaded varieties in this group showed severe to very severe mottling, stunting, leaf distortion, and chlorosis—combined effects of both viruses but markedly predominant with virus A. The general chlorosis was yellow, a color resulting from the severe mottling. The ballheads likewise expressed a marked predominance of virus A symptoms, resulting in a severe or very severe leaf distortion, stunting, and necrosis. Necrosis was markedly more severe in these varieties than in others. In general, the viruses produced a yellow-green mottling in the ballhead varieties in contrast to the yellow mottling produced in the roundheaded varieties.

TABLE 5.—*Type and intensity of symptoms expressed by 18 cabbage varieties infected with mosaic*

[0, none; +, slight; ++, moderate; +++, severe; ++++, very severe]

Variety	Immature-head stage							Seed stage						
	Mottling	Vein clearing and vein banding	General chlorosis	Stunting	Leaf distortion	Defoliation	Necrosis	Mottling	Vein clearing and vein banding	General chlorosis	Stunting	Leaf distortion	Defoliation	Necrosis of leaves, stalk, and pods
Wisconsin All Seasons	+	++	++	+	+	0	0	+	++	++	+	+	++	+
Stein's Early Flat Dutch	+	++	++	+	+	0	0	++	++	++	+	+	++	+
All Head Select	+	++	++	+	+	0	0	+	+++	+++	+	+	++	+
Globe	++	+	++	++	+	0	0	++	++	++	+	+	++	+
Early Jersey Wakefield	++	+++	++	++	++	+	+	++	+++	+++	+	+	++	++
Premium Late Flat Dutch	++	+++	+++	+++	+++	+	++							
Glory of Enkhuizen	++++	+	+++	+++	+++	+	+	++++	+	+++	++	++	+++	++
Charleston Wakefield	+++	+++	+++	+++	+++	+	++	+++	++	+++	++	+++	++++	+++
Early Round Dutch	++++	++	+++	+++	+++	+	++	++++	+	+++	++	++	++++	+++
Resistant Detroit	++++	+	+++	+++	+++	+	++	++++	+	+++	++	++	++++	+++
Golden Acre	++++	+	+++	+++	+++	+	++	++++	+	+++	++	++	++++	+++
Marion Market	++++	+	+++	+++	+++	+	++	++++	+	+++	++	++	++++	+++
All Head Early	++	++++	++++	+++	+++	+	++							
Copenhagen Market	++++	+	+++	+++	+++	+	++	++++	+	+++	+++	++	++++	+++
Wisconsin Hollander	++++	++	+++	+++	+++	++	+++	++	+++	++++	+	+	+++	++
Penn State Ballhead	++++	+	++	++++	++++	++	++++	+++	+	++	+++	+	++++	++++
Danish Ballhead	++++	+	++	++++	++++	++	++++	+++	+	++	+++	+	++++	++++
Ferry's Hollander	++++	+	++	++++	++++	++	++++	+++	+	++	+++	+	++++	++++

In the seed stage the varieties showed the same general type of symptoms as in the immature-head stage, but necrosis and defoliation were generally much more severe.

Effect of Air Temperatures on Symptoms

Pound and Walker (*4*) showed that symptoms of virus B are most pronounced at low temperatures and those of virus A at high temperatures. Furthermore, plants infected with a combination of the viruses may change symptom types if the air temperatures under which they are growing change. The cabbage seed crops in the Puget Sound section offered excellent opportunity for further observations on the effect of temperature. Plants there are set out in late summer and remain in the open throughout the winter, bolting to produce seed-stalks in early spring. Thus in 1943–44 plants grew through a season in which the temperature gradually fell from a mean of 60.6° F. in September to one of 38.4° in December and then rose to a mean of 62.0° in June.

Although air temperatures in the Puget Sound section never reach a point high enough to cause the suppression of virus B symptoms and the best expression of those of virus A, there were marked changes in type as temperature changes occurred. In early fall symptoms of virus A were quite conspicuous and those of virus B became increasingly so until winter dormancy stopped growth. In April and early May, when plants had resumed growth and temperatures were still low, virus B symptoms were markedly predominant over those of virus A. This was especially true in those varieties most susceptible to virus B. During late fall and winter enation development, characteristic of virus B, was pronounced. The roundheaded and ballhead types, although showing a predominance of virus B symptoms, also showed conspicuous mottling or chlorotic ringing. By June virus A symptoms attained prominence and plants showed severe mottling, necrosis, and defoliation.

EXPERIMENTAL PLOTS IN 1944 AND 1945

Summer of 1944

Twenty different varieties were transplanted in paired rows in 4 randomized replicates, and the plants were inoculated 2 weeks later with a mixture of viruses A and B from Wisconsin. Each replicate row contained 50 plants. Varieties were rated as to their susceptibility by a disease index; notes were taken 60, 80, and 110 days after transplanting. Results very similar to those of 1943–44 were obtained (table 6). Wisconsin All Seasons, Stein's Early Flat Dutch, All Head Select, Succession, and Globe headed the list as the least susceptible class. Wisconsin Hollander, Bugner, and Penn State Ballhead showed only moderate symptoms until late in the season, but at the time of maturity the symptoms had become so severe that they were hardly less marked than those of the other ballheads. Ferry's Hollander and Wisconsin Ballhead, which are very similar in type and time of maturity, were conspicuously the most susceptible varieties. Stunting, leaf distortion, defoliation, mottling, and necrosis were very severe in these 2 varieties. All the roundheaded varieties except

TABLE 6.—*Relative susceptibility of 20 cabbage varieties to cabbage mosaic, 1944*

Variety	Disease-index value at indicated period after transplanting[1]		
	60 days	80 days	110 days
Wisconsin All Seasons	29.8	36.8	.....
Stein's Early Flat Dutch	30.8	42.6	.....
All Head Select	31.6	39.9	.....
Succession	36.1	45.1	.....
Globe	37.9	45.9	.....
Jersey Queen	45.6	[2]	.....
Wisconsin Hollander	45.7	51.7	62.4
All Head Early	46.1	55.4	.....
Charleston Wakefield	48.8	53.7	.....
Bugner	48.8	52.1	64.2
Penn State Ballhead	50.6	53.7	65.0
Marion Market	51.2	57.1	.....
Early Round Dutch	52.2	58.6	.....
Copenhagen Market	52.7	[2]	.....
Golden Acre	52.7	[2]	.....
Midseason Market	53.1	59.6	.....
Glory of Enkhuizen	54.2	59.4	.....
Premium Late Flat Dutch	57.6	59.6	.....
Ferry's Hollander	58.6	64.4	71.2
Wisconsin Ballhead	59.2	64.1	69.4
Difference required for significance (19:1)	3.7	3.8	.....

[1] Values are averages of 4 replications of 50 plants each. [2] Harvested.

Globe were very similar in their reaction. Severe yellow mottling, leaf distortion, and necrosis were the characteristic symptoms.

SUMMER OF 1945

In 1945 it was decided to broaden the scope of the tests by using more virus combinations. Sixteen varieties of cabbage were transplanted in paired rows in three randomized replicates. Each replicate contained a row of each variety inoculated with a mixture of isolates of viruses A and B occurring in the Puget Sound section, one inoculated with a mixture of viruses A and B isolates from Wisconsin, and one inoculated with a mixture of the California black ring virus and Wisconsin virus B. This study was designed to test further varietal reactions as well as to differentiate further the virus isolates tested. Each plant was placed in one of four severity classes for three different symptom categories designated as stunting and leaf distortion, mottling and chlorosis, and necrosis. The results of this experiment are shown in table 7.

When the symptom types in the three categories were considered together no significant differences were found in the severity of the three strains of turnip virus 1. The viruses had almost identical over-all average disease-index values. Moreover, the varieties had somewhat similar positions of relative susceptibility with each virus, although some differences did occur. Furthermore, the varieties used during the previous two seasons again showed approximately the same relative susceptibility. Stein's Early Flat Dutch, All Head Select, Wisconsin All Seasons, Succession, and Globe showed the least severe symptoms. The other roundheaded varieties were intermediate in susceptibility. Improved Wisconsin Ballhead and Ferry's Hollander were the most severely affected. Improved Wisconsin

TABLE 7.—*Relative susceptibility of 16 cabbage varieties to mosaic viruses when stunting and leaf distortion, mottling and chlorosis, and necrosis were all considered and when necrosis only was considered, 1945*

[Values are averages of 3 replicates; notes taken 80 days after transplanting except for values in parentheses, which are based on ratings made 120 days after transplanting]

Variety	Disease-index value based on 3 symptom categories and the indicated mixture of viruses			Disease-index value based on necrosis only and the indicated mixture of viruses		
	Wisconsin A+B	Washington A+B	Black ring and Wisconsin B	Wisconsin A+B	Washington A+B	Black ring and Wisconsin B
Stein's Early Flat Dutch	42.1	45.8	44.6	43.8	43.1	49.0
All Head Select	43.2	44.5	46.1	45.9	49.3	54.1
Wisconsin All Seasons	43.4	47.9	44.1	45.1	46.5	51.1
Succession	46.3	48.9	50.0	42.4	42.5	58.3
Globe	47.4	49.1	51.3	52.6	57.1	61.7
Charleston Wakefield	49.3	50.6	51.4	42.3	49.5	54.3
Penn State Ballhead	49.9 (71.2)	45.8 (72.2)	48.9 (69.6)	47.8	43.5	52.9
Bugner	50.2 (71.8)	49.4 (64.6)	45.0 (64.7)	54.2	40.9	48.5
Premium Late Flat Dutch	52.5	55.4	54.1	51.1	52.5	56.8
Jersey Queen	52.7	52.9	54.1	51.9	48.3	58.6
Wisconsin Hollander	54.0 (69.2)	49.4 (72.4)	47.1 (68.7)	42.4	45.9	50.8
Glory of Enkhuizen	56.7	61.3	60.6	49.5	56.6	58.6
Copenhagen Market	58.4	55.6	55.4	54.5	52.2	56.7
Marion Market	59.2	53.5	58.2	55.5	53.9	55.8
Improved Wisconsin Ballhead [1]	59.8 (75.6)	55.7 (73.7)	58.1 (78.8)	44.4	48.7	49.5
Ferry's Hollander	71.3 (79.3)	75.4 (82.0)	65.3 (80.4)	60.4	63.3	57.1
Average	52.3	52.6	52.1	49.0	49.6	54.6
Difference required for significance (19:1)	3.8	4.1	5.2	8.5	6.4	[2]

[1] A different and more tolerant selection of Wisconsin Ballhead than that used previously.
[2] Differences not significant.

Ballhead, which is a selection from a cross between the original Wisconsin Ballhead and Wisconsin Hollander, exhibited much greater tolerance than the Wisconsin Ballhead used the previous season. Penn State Ballhead, Bugner, and Wisconsin Hollander again showed only moderately severe symptoms until they had reached maturity, when the symptoms became very severe. These varieties apparently have an early-season tolerance which breaks in late season.

When necrosis was the only disease criterion the black ring virus was markedly more severe than the two isolates of virus A. This is indicated by the fact that when necrosis only was considered, the index value of the black ring virus was 4.8 percent more than when all three symptom categories were considered, whereas the value of the virus A isolates was 6 percent less. Thus, the value of the black ring virus was 11 percent greater than that of the virus A isolates. That the black ring virus is a more necrotic virus than virus A was reported before (*4*). The very close agreement of the virus A isolates is interesting. The identities of these two isolates, as well as those of the Wisconsin and Washington isolates of virus B, have been proved by extensive greenhouse tests. When only necrosis was considered there were significant differences in the varietal reactions with the virus A isolates but not with the black ring virus. The relative severity of necrosis compared with other symptom types on specific varieties was indicated by the rise or fall in the index value when necrosis was the only criterion used.

By studying the disease-index values calculated on the basis of necrosis only, it can be seen that greater variation occurred than when all three groups of symptom types were used; the varieties did not occupy the same relative positions. This increased variability is evidenced by the difference in error variances of the two different calculations as shown in table 8.

TABLE 8.—*Error variances for analyses of varietal susceptibility for disease-index values based on 3 groups of symptom types and on necrosis only*

Viruses	Error variance for analysis of—	
	3 groups of symptom types	Necrosis only
Wisconsin A+B	5.31	25.78
Washington A+B	6.14	14.93
Black ring and Wisconsin B	9.62	29.77

DISCUSSION

Only a rough quantitative measurement of the severity of virus symptoms can be made by a disease-severity rating system. However, it is believed that sufficient evidence is presented herein to show that there are marked differences in the susceptibility of cabbage varieties to mosaic viruses.

The varieties manifesting the most resistance to cabbage mosaic are Wisconsin All Seasons, Stein's Early Flat Dutch, All Head Select, Succession, and Globe. All of these except Globe are of the same type (flathead, late midseason). The first four named are very similar in type and time of maturity; of these Wisconsin All Seasons and All Head Select are yellows-resistant. All Head Select is markedly more resistant to mosaic than All Head Early, from which it was derived. Moreover, All Head Select resembles Wisconsin All Seasons and Stein's Early Flat Dutch more than it does All Head Early. Globe and Succession are more susceptible than the others in this group. Globe, which is yellows-resistant, is markedly more resistant than Glory of Enkhuizen, from which it was derived. All of the varieties in this most resistant group characteristically show general chlorosis, vein clearing, and slight mottling, and there is no extreme leaf distortion here as is characteristic of the more susceptible varieties. In general, the effects of cabbage virus B predominate over those of virus A.

Early Jersey Wakefield, Charleston Wakefield, Golden Acre, Resistant Detroit, Copenhagen Market, Marion Market, Midseason Market, Glory of Enkhuizen, Early Round Dutch, All Head Early, and Premium Late Flat Dutch occupied an intermediate position of susceptibility. Early Jersey Wakefield and Charleston Wakefield were usually less susceptible than the others in this group. All roundheaded varieties tested showed almost identical reactions. No difference was noticeable between Resistant Detroit (yellows-resistant) and Copenhagen Market (yellows-susceptible). All roundheaded varieties of this group showed severe or very severe yellow mottling, severe leaf distortion, severe stunting, and necrosis. Early Jersey Wakefield,

Charleston Wakefield, All Head Early, and Premium Late Flat Dutch showed much more pronounced effects of virus B (vein clearing, vein banding, and general chlorosis) than did any other varieties studied. These varieties were especially good indicators for this virus.

In the most susceptible group are such varieties as Ferry's Hollander, Wisconsin Ballhead, Improved Wisconsin Ballhead, Penn State Ballhead, Wisconsin Hollander, and Bugner. However, the last three varieties mentioned were only moderately susceptible through midseason and showed no worse effects than the roundheads at the time the latter were mature. By the time the ballheads were mature, however, they showed very severe symptoms. This would indicate that these varieties were more susceptible than the roundheads because of a longer growing period. This is not true, however, of Ferry's Hollander and Wisconsin Ballhead, which were most severely affected throughout the season. These two varieties were by far the most susceptible ones studied. Improved Wisconsin Ballhead is distinctly more resistant than the original Wisconsin Ballhead. Although the symptoms on all varieties were the combined effects of viruses A and B, those on the ballheads were predominantly those of virus A (coarse mottling, leaf distortion, and necrosis). The mottling was yellow green instead of yellow as on the roundheaded varieties.

The relative susceptibility of the various varieties was about the same whether the cabbage A strain or cabbage black ring strain of turnip virus 1 was combined with the cabbage B strain of cauliflower virus 1 to produce the mosaic disease. However, the black ring virus produced more necrosis but less mottling and leaf distortion than virus A. When the over-all disease severity was considered the two viruses were equally severe; when only necrosis was considered, the black ring virus was the more severe.

That the viruses causing cabbage mosaic in the Puget Sound section are identical with those occurring in the Midwest rather than with the cabbage viruses occurring in California is shown by this investigation and by extensive greenhouse tests not reported herein.

SUMMARY

In a survey of the relative susceptibility of certain cabbage varieties to mosaic viruses it was found that the varieties represented a distinct gradient but fell roughly into three classes of susceptibility. The least susceptible group includes Wisconsin All Seasons, Stein's Early Flat Dutch, All Head Select, Succession, and Globe. The group of intermediate susceptibility comprises Golden Acre, Resistant Detroit, Copenhagen Market, Marion Market, Midseason Market, Glory of Enkhuizen, Early Round Dutch, Early Jersey Wakefield, Charleston Wakefield, All Head Early, and Premium Late Flat Dutch. In the most susceptible group are Wisconsin Hollander, Bugner, Penn State Ballhead, Ferry's Hollander, Wisconsin Ballhead, and Improved Wisconsin Ballhead. The first three listed in this group show only moderately severe symptoms until late season, when they break and show very severe effects of the disease. When the over-all severity is considered the varieties occupy about the same position of relative susceptibility whether cabbage virus A or the cabbage black ring virus is combined with virus B to produce the mosaic disease.

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BEET MOSAIC IN THE PACIFIC NORTHWEST[1]

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INTRODUCTION

For several years mosaic has been a serious disease of garden beets (*Beta vulgaris* L.) grown for seed in the Puget Sound section of Washington. In 1931 Jones (*8*)[3] reported the finding of seed fields in which 100 percent of the plants were infected. The system of culture used in the past few years has tended to increase the severity of mosaic. Serious reductions in seed yield have resulted and, in consequence, the acreage of garden beets for seed has gradually declined even though prices have risen during the period. Because of wartime demands for increased seed production a disease-investigation program was undertaken in this important seed-producing section. This paper is a report of a study of the occurrence, host range, symptoms, and properties of the virus causing mosaic.

MATERIALS AND METHODS

The culture of the beet mosaic virus tested was obtained in 1943 from an infected seed plant of garden beet. Symptoms, host range, and properties were studied in a greenhouse which was fumigated frequently to control insects. Air temperatures were maintained at 60° to 70° F. Stock cultures of the virus were carried in plants of mangel (*Beta vulgaris*) growing in insectproof cages. Frequent inoculations of healthy plants were made to provide inoculum for study. Plants used in the experiments were grown in greenhouse compost in 4-inch clay pots. Mechanical inoculations were made by sprinkling the plants with powdered carborundum and then sponging them with small bits of absorbent cotton dipped in sap extract from stock-virus plants. Insect transmission was carried out by allowing nonviruliferous aphids to feed on diseased plants for 24 to 48 hours and then transferring them by means of a camel's-hair brush to a small bit of paper. This paper was then placed on healthy plants to which the aphids moved to feed.

[1] Received for publication September 11, 1946. Cooperative investigation by Bureau of Plant Industry, Soils, and Agricultural Engineering and the Washington Agricultural Experiment Stations at the Vegetable Seed Production Laboratory, Mount Vernon, Wash. Scientific Paper No. 672 of the State College of Washington and Washington Agricultural Experiment Stations.

[2] The writer expresses sincere appreciation to Neil Allan MacLean for assisting in this study.

[3] Italic numbers in parentheses refer to Literature Cited, p. 41.

Properties of the virus were determined according to methods recently described (*17*). In making the host-range study, at least five plants were inoculated in each test and an equal number of uninoculated plants were kept as controls. Most tests were repeated one or more times. The presence or absence of the virus in each test was confirmed by mechanical transfer from the host in question to test plants which produced characteristic symptoms.

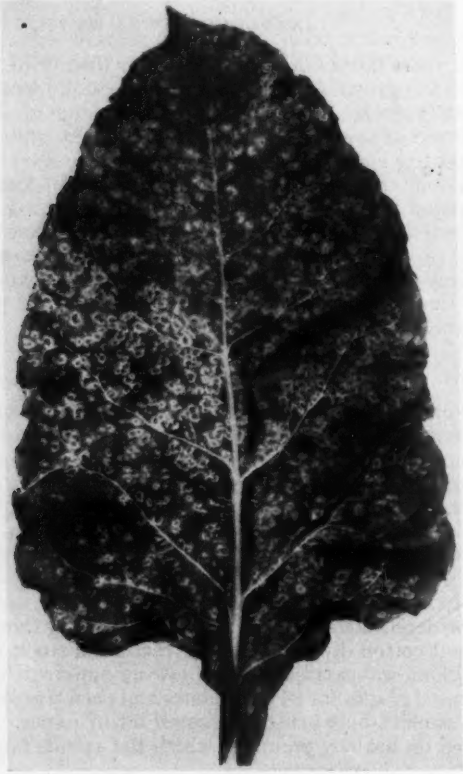

FIGURE 1.—Systemic symptoms produced on leaf of Danish Sludstrup mangel by the beet mosaic virus. Note mottling and chlorotic ringing.

EXPERIMENTAL RESULTS

SYMPTOMS AND HOST RANGE

In the field the symptoms of beet mosaic may assume a multiplicity of forms. Mangel, sugar beet (*Beta vulgaris*), and chard (*B. vulgaris* var. *cicla* L.) characteristically show a conspicuous mottling with chlorotic rings and ring spots that become zonate and frequently necrotic with age (fig. 1). These ring spots usually consist of yellow rings with dark-green centers. On older leaves the lesions may

develop in a conspicuous pattern of concentric ringing with reddish-brown pigmentation. On garden beet the symptoms usually appear as a conspicuous vein clearing followed by numerous small chlorotic rings with pigmented centers or as solid chlorotic spots with pigmented peripheries (fig. 2, *A*, *C*). Ring spotting may be entirely absent from some plants, a chlorotic mottling being the prevalent symptom. Often young leaves show a conspicuous, irregularly etched pattern along the veins (fig. 2, *B*). The concentric ringing, consisting of alternately pigmented and light areas, is very characteristic and may occur with pigmented or light centers. Infected plants usually develop an excessive amount of anthocyanin pigment and are thus easily detected in a field. With age the seed plants may develop necrosis, which is followed by severe leaf abscission. Leaves of infected plants often become very coriaceous. Stunting and distortion of leaves are very pronounced.

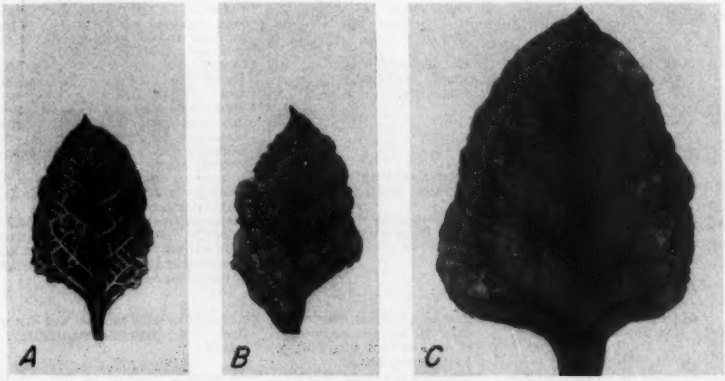

FIGURE 2.—Systemic symptoms of beet mosaic on leaves of garden beet: *A*, Chlorotic vein clearing; *B*, etched pattern along veins; *C*, pigmented rings and ring spots.

In table 1 are given the symptoms produced on various hosts in greenhouse tests. It will be noted that the virus was rather restricted in its host range, as in families other than Chenopodiaceae and Amaranthaceae it infected only chickweed, aster, zinnia, shepherds-purse, verbena, pansy, *Iodanthus pinnatifidus* (Michx.) Steud., and New Zealand spinach among the 68 species inoculated. In nature beet mosaic occurs very widely on *Amaranthus retroflexus* L., affecting a relatively high percentage of the plants in the seed-growing section. It also occurs widely on zinnia, usually together with a strain of cucumber virus 1 and a strain of turnip virus 1. The beet mosaic virus has been isolated frequently from lambsquarters, and it commonly infects spinach seed plants. It is thought that in addition to *Beta vulgaris*, spinach, zinnia, and *Amaranthus* are the primary natural hosts. It is of interest that this virus was infectious to shepherds-purse and *Iodanthus* but not to other cruciferous plants tested. Hoggan (*7*) was able to obtain local symptoms on tobacco with the sugar-beet mosaic virus by aphid transmission but not by mechanical transmission. In the present study aphid transmission

to this host was not attempted, but mechanical transmission tests were negative.

TABLE 1.—*Symptoms produced on various species inoculated mechanically with the beet mosaic virus in the greenhouse at 60° to 70° F.*

Family and plant	Local symptoms	Systemic symptoms
AIZOACEAE:		
Tetragonia expansa Murr. (New Zealand spinach).	Small, pin-point, necrotic lesions with conspicuous yellow halo.	None; virus recovered only from inoculated leaves.
AMARANTHACEAE:		
Amaranthus retroflexus L. (pigweed).	Small, necrotic lesions in 10 to 14 days.	Conspicuous vein clearing and fine mottling, leaving young leaves severely stunted and distorted.
CARYOPHYLLACEAE:		
Stellaria media (L.) Cyrill. (chickweed).	None	Small but conspicuous, chlorotic lesions with marked leaf distortion; chlorotic vein clearing; stunting.
CHENOPODIACEAE:		
Atriplex hortensis L. (orach).	Numerous, small, chlorotic rings, soon becoming tan and necrotic (parchment-like).	Numerous, small, whitish dots developing into a yellow mottling; yellow vein clearing; marked curling of leaf laminae and petioles; old leaves becoming necrotic and dying.
Beta vulgaris L. (garden beet, vars. Detroit Dark Red, Wonder).	Few pigmented lesions or small, pigmented rings or ring spots.	Marked chlorotic vein clearing followed by an irregular ring spotting and etched pattern; young leaves severely stunted and distorted, curling along the margins; yellow-green mottling and marked reddening developing with age; root development severely suppressed.
Beta vulgaris var. *cicla* L. (Swiss chard, var. Lucullus).	Scattered, chlorotic lesions, 1 to 2 mm. in diameter.	Conspicuous vein clearing followed by chlorotic mottling; marked stunting and distortion of leaves.
Beta vulgaris (mangel, vars. Danish Sludstrup, Golden Tankard).	As described for Swiss chard; sporadic in occurrence.	Conspicuous vein clearing followed by chlorotic mottling; young leaves show extensive chlorotic areas at leaf apex; marked stunting and distortion of young leaves.
Beta vulgaris (sugar beet, var. Klein Wanzleben).	do	Do.
Chenopodium album L. (lambsquarters).	Numerous, distinct, pin-point, chlorotic lesions, consisting of tan centers surrounded by chlorotic halos.	Fine mottling with marked leaf distortion; very severe stunting; lethal in 3 weeks.
Spinacia oleracea L. (spinach, vars. Nobel, Viking, Virginia, Savoy, Old Dominion, Bloomsdale, King of Denmark, Hollandia, Giant Thick-Leaved).	Numerous, pin-point, chlorotic lesions, enlarging slightly and becoming golden yellow; sometimes becoming necrotic.	Vein clearing followed by fine yellow mottling with severe stunting and distortion of young leaves; many plants reduced to conspicuous rosette; lethal to many plants.
COMPOSITAE:		
Aster amellus L. (aster)	None	Fine mottling composed of numerous, small, chlorotic dots.
Zinnia elegans Jacq. (zinnia, var. Lilliput).	do	Mild, diffuse, yellow mottling; slight stunting and distortion of leaves.
CRUCIFERAE:		
Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	Small, chlorotic lesions becoming necrotic and zonate.	Mild, fine, chlorotic mottling.
Iodanthus pinnatifidus (Michx.) Steud. (*Thelypodium pinnatifidum*).	None	Marked chlorotic vein clearing and fine mottling.
VERBENACEAE:		
Verbena hybrida Voss (garden verbena).	do	None; virus recovered in moderate concentration.
VIOLACEAE:		
Viola tricolor L. var. *hortensis* DC. (pansy).	do	Do.

On spinach the beet mosaic virus produced both local and systemic symptoms that were easily confused with those produced by turnip virus 1 and a strain of cucumber virus 1. Symptoms produced on spinach, *Amaranthus*, lambsquarters, and New Zealand spinach are shown in figure 3.

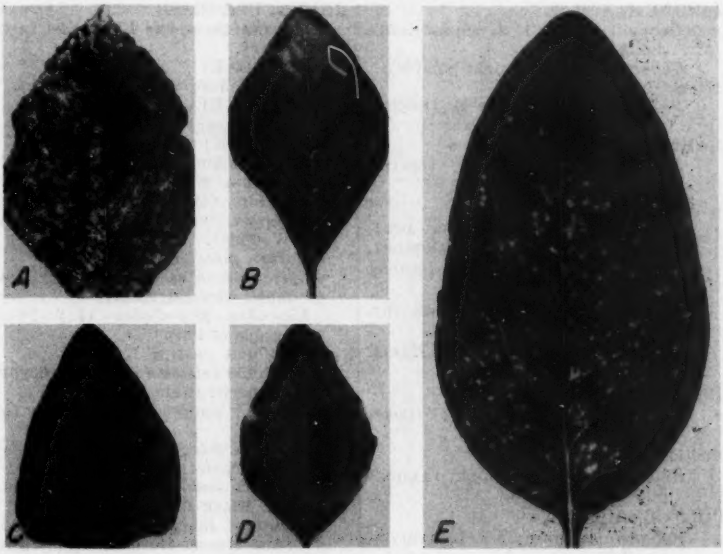

FIGURE 3.—Symptoms produced by the beet mosaic virus on selected greenhouse hosts: *A*, Systemic symptoms on leaf of *Amaranthus retroflexus; B*, local symptoms on the same host; *C*, local chlorotic lesions on *Tetragonia expansa; D*, local chlorotic lesions on leaf of *Chenopodium album; E*, systemic symptoms on *Spinacia oleracea*, var. Hollandia.

No infection was obtained on the following species in mechanical inoculation tests:

CAMPANULACEAE:
- *Campanula medium* L. (canterbury-bells).

CARYOPHYLLACEAE:
- *Dianthus barbatus* L. (sweet-william).

COMPOSITAE:
- *Calendula officinalis* L. (calendula, var. Orange King).
- *Centaurea nigra* L. (knapweed).
- *Centaurea repens* L. (Russian knapweed).
- *Chrysanthemum morifolium* Ram. (chrysanthemum).
- *Cirsium arvense* (L.) Scop. (Canada thistle).
- *Dahlia pinnata* Cav. (dahlia).
- *Helianthus annuus* L. (sunflower).
- *Lactuca sativa* L. (lettuce, var. Iceberg).
- *Tagetes patula* L. (French marigold).

CRUCIFERAE:
- *Brassica juncea* (L.) Cosson (Indian mustard).
- *Brassica oleracea* var. *capitata* L. (cabbage, var. Jersey Queen).

CRUCIFERAE—Continued
- *Brassica oleracea* var. *gemmifera* (DC.) Zenker (brussels sprouts, var. Long Island Mammoth).
- *Brassica oleracea* var. *gongylodes* L. (kohlrabi, var. Early White Vienna).
- *Brassica pekinensis* (Lour.) Rupr. (Chinese cabbage, var. Wong Bok).
- *Cheiranthus cheiri* L. (wallflower).
- *Hesperis matronalis* L. (dames violet).
- *Lepidium virginicum* L. (wild pepperpergrass).
- *Lobularia maritima* (L.) Desv. (sweet alyssum).
- *Malcomia maritima* (L.) R. Br. (Virginian stock).
- *Matthiola incana* var. *annua* (L.) Voss (annual stock, var. Dwarf Large Flowering Ten Weeks).
- *Neslia paniculata* (L.) Desv. (ball-mustard).
- *Raphanus sativus* L. (radish, var. Early Scarlet Globe).
- *Thlaspi arvense* L. (pennycress).

CUCURBITACEAE:
- *Cucumis sativus* L. (cucumber, var. Long Green).
- *Cucurbita pepo* L. (sugar pumpkin).

DIPSACACEAE:
- *Scabiosa atropurpurea* L. (mourning bride).

LABIATAE:
- *Salvia splendens* Ker. (scarlet sage).

LEGUMINOSAE:
- *Lathyrus odoratus* L. (sweet pea).
- *Lupinus pubescens* Benth. (lupine).
- *Phaseolus coccineus* L. (scarlet runner bean).
- *Phaseolus vulgaris* L. (bean, var. Kentucky Wonder).
- *Vigna sinensis* (Tarner) Hassk. (cowpea, var. Blackeye).

PHYTOLACCACEAE:
- *Phytolacca americana* L. (pokeweed).

POLEMONIACEAE:
- *Phlox drummondii* Hook. (annual phlox).

POLYGONACEAE:
- *Polygonum hydropiper* L. (smartweed).
- *Polygonum scandens* L. (false buckwheat).
- *Rumex occidentalis* S. Wats. (dock).

RANUNCULACEAE:
- *Delphinium ajacis* L. (rocket larkspur).

RESEDACEAE:
- *Reseda odorata* L. (mignonette).

SCROPHULARIACEAE:
- *Digitalis purpurea* L. (foxglove).

SOLANACEAE:
- *Atropa belladonna* L. (belladonna).
- *Capsicum frutescens* var. *cerasiforme* (Mill.) Bailey (cherry pepper, var. Early California Wonder).
- *Datura metel* L.
- *Lycopersicon esculentum* Mill. (tomato, var. Earliana).
- *Nicandra physalodes* (L.) Pers. (apple-of-Peru).
- *Nicotiana rustica* L.
- *Nicotiana tabacum* L. (tobacco, var. Connecticut Havana 38).
- *Physalis pubescens* L. (husk tomato).
- *Solanum integrifolium* Poir. (Chinese scarlet eggplant).
- *Solanum melongena* L. (eggplant, var. Black Beauty).
- *Solanum tuberosum* L. (potato, vars. Russet Burbank and White Rose).

UMBELLIFERAE:
- *Daucus carota* L. (carrot, var. Red Cored Chantenay).

PROPERTIES

In determining the physical properties of the beet mosaic virus, Danish Sludstrup mangel was used as the inoculation test plant and as the source of inoculum. Mangel rather than garden beet was used because of the absence of anthocyanin pigmentation, which is so common in the latter. The properties of the virus are given in table 2.

The inactivation points were dilution tolerance 1 to 2,000, thermal inactivation 61° C. for 10 minutes, and aging in vitro 72 hours. These agreed very closely with those given by Hoggan (*7*). In view of the properties and symptoms described, there seems no doubt that this virus is identical with that described as causing sugar-beet mosaic in this country (*7*, *8*, *12*). Throughout the studies no evidence of variability in the symptoms or host range of this virus was seen.

Verplancke (*16*) reported the physical properties of the beet mosaic virus in Belgium as follows: Thermal inactivation 90° to 95° C. for 10 minutes, dilution tolerance 1 to 100,000, and aging in vitro at room temperature 144 hours. It is obvious that the virus used by Verplancke was not the same as that used in this study or as the one used by Hoggan (*7*).

TABLE 2.—*Properties of beet mosaic virus as determined by the number of inoculated mangel plants that became infected*

[10 plants inoculated in each trial]

Type and degree of treatment	Plants infected with beet mosaic virus			
	First trial	Second trial	Third trial	Fourth trial
Dilution:	*Number*	*Number*	*Number*	*Number*
0	10	10	10	10
1–10	9	9	10	4
1–100	3	2	4	3
1–500	1	1	1	0
1–1,000	1	1	0	0
1–2,000	0	0	0	0
Aging in vitro (hours at 20° C.):				
0	10	10	5	10
24	2	2	3	5
48	0	0	0	3
72	0	0	0	0
Heating for 10 minutes (°C.):				
0	10	10	10	10
50	10	8	9	8
51	8	9	8	8
52	10	10	9	8
53	8	8	8	3
54	8	10	7	5
55	9	10	3	5
56	2	7	2	2
57	3	6	2	2
58	5	4	1	0
59	0	1	0	1
60	0	0	1	0
61	0	0	0	0

TRANSMISSION

The virus was readily transmissible by mechanical means with or without powdered carborundum as an abrasive. Hoggan (*7*) and Rawlins and Tompkins (*11*) also were able to transmit by mechanical means the virus of sugar-beet mosaic occurring in this country. Earlier Robbins (*12*) and Jones (*8*) had reported negative results in mechanical transmission tests. It seems probable that techniques used rather than variability in the virus explain the differences in results obtained in mechanical transmission tests.

No evidence was found that would indicate any reduction in activity of the virus in sap obtained by macerating spinach or beet tissue as has been reported for other viruses affecting these hosts (*9*).

Transmission was readily obtained with the black bean aphid (*Aphis fabae* Scop.). It is a common occurrence for this aphid to develop abundantly in beet seed fields and on wild chenopodiaceous hosts during midsummer and to migrate thence into young steckling beds. It is thought that this aphid is the primary vector among chenopodiaceous hosts. The green peach aphid (*Myzus persicae* (Sulz.)) was also found to be a vector, and it is probable that spread to nonchenopodiaceous hosts occurs by this vector. Transmission was also obtained by the cabbage aphid (*Brevicoryne brassicae* (L.)); but since it is difficult to obtain colonization of this aphid on beet and spinach, it is thought that this aphid is not a common vector.

In 1943 a large sample of garden beet seed (var. Asgrow Wonder) was collected from plants known to be infected with the mosaic virus. Over 5,000 seedlings were grown from this seed in steam-sterilized soil, and after 4 weeks' growth no evidence of mosaic infection was found. The virus was isolated, however, from semimature seed by macerating the seed in a small amount of water and inoculating healthy plants. Similar tests with well-matured seed were negative.

Workers with the sugar-beet mosaic virus occurring in continental Europe and England have reported varying results from mechanical and seed transmission tests with the virus. Verplancke (*15*) was repeatedly successful in mechanically transmitting the virus in Belgium. Smith (*14*), in his studies of the virus in England, obtained mechanical transmission from beet to beet and from beet to lambsquarters. Böning (*1*), Böning and Schaffnit (*2*), and Schmidt (*13*), on the other hand, were unable to transmit mechanically the virus causing mosaic in Germany. Ducomet (*4*) reported positive results from seed transmission tests, and Verplancke (*15*) was able to show 7.1 percent transmission in seed from mosaic-infected plants. Gram (*6*), however, reported that transmission through seed had not been observed in Denmark. Smith (*14*) stated that there was no evidence that more than one virus was involved in the mosaic in these areas.

CONTROL

The method that has been followed in the Puget Sound section for the production of beet seed has been ideal for a continuous perpetuation of mosaic. Steckling beds have been planted indiscriminately in relation to maturing seed fields and have often been adjacent to them. There is an overlapping period of almost 3 months from the time of sowing seed to harvesting seed fields.[4] Aphid migration from seed fields to steckling beds during this period has resulted in very widespread infection of seedling plants. Infection of as high as 100 percent of the plants has been the rule rather than the exception. This condition is identical with that for mosaic of seed plants of cabbage in the Puget Sound section (*10*). In 1931 Jones (*8*) pointed out the fallacy of such cultural practices in production of beet seed.

Recommendations to locate steckling beds in areas isolated from diseased fields have resulted in fair to good control of beet mosaic, but the results have been much less satisfactory than those reported for cabbage mosaic (*10*). In table 3 are given the results obtained in 1944–45. Very similar results were obtained in 1943–44. These data show that, even though the isolation of steckling beds used during these two seasons did not completely prevent the disease, practical control was obtained.

[4] Seed is sown in steckling beds during June. In late fall the roots are taken up and placed in pit storage for the winter. The following April they are transplanted to seed rows, and during August and September seed is harvested. Although secondary infections occur after transplanting in the spring, the disease initiated at this time is much less serious than that initiated during the seedling stage.

TABLE 3.—*Effect of isolation of steckling beds on amount of mosaic on plants in seed fields of garden beet, mangel, and chard, 1944–45*

[Each value based on sample of 600 plants]

Degree of isolation of steckling beds and crop	Field No.	Plants infected
		Percent
Excellent (more than 5 miles):		
Mangel	1	1
Chard	[1] 2	1
Garden beet	3	3
Do	4	5
Mangel	5	5
Do	6	6
Garden beet	7	14
Do	8	21
Mangel	[1] 9	21
Do	[1] 10	26
Poor (less than 1 mile):		
Mangel	[2] 11	18
Do	[2] 12	45
Garden beet	13	55
Chard	14	58
Mangel	15	98
Do	16	99
Very poor (less than ¼ mile):		
Chard	17	60
Garden beet	18	94
Do	19	97
Mangel	20	98
Do	21	100
Do	22	100

[1] Fields 2, 9, and 10 were from beds at the same location. Note that chard which was seeded much later than mangel had much less infection.
[2] Fields 11 and 12 came from 2 beds side by side, but the bed for field 12 was seeded 2 weeks earlier than that for field 11.

Four of the fields (7, 8, 9, and 10) were in the same location more than 20 miles from the nearest known beet seed field. The moderate infection found seems to indicate rather widespread occurrence of the virus among wild host plants or a wide range of dissemination of the aphid vector. Efforts to find wild host plants infected with the beet virus in this section, however, were unsuccessful. Cabbage plants grown in the same location and having the same degree of isolation from cabbage seed fields escaped infection with cabbage mosaic almost entirely. Fields 1, 3, 5, and 6, which had the same degree of isolation from the seed-growing section but in different directions, were much less infected.

DISCUSSION

The mosaic of beet described in this paper is the most important disease of beet seed crops in the Puget Sound section. It has also been reported as severe in the Sacramento Valley of California,[5] and the writer has seen it in seed crops in the Willamette Valley of Oregon. Brewbaker (*3*) and Gaskill (*5*) showed losses of seed yields of sugar beet due to mosaic in Colorado and Idaho up to 31.3 percent and 39 percent, respectively. The writer is of the opinion that losses in seed crops of the garden beet in the Puget Sound section often run well over 50 percent. Many seed plants are so severely stunted by the disease that yields are very low. Observations lead the writer to believe that reductions in seed yields are much greater for garden beet than for mangel, sugar beet, or chard. Symptoms produced in

[5] BARNETT, H. L. VEGETABLE DISEASES IN CALIFORNIA. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 28: 447–449. 1944. [Processed.] (See p. 447.)

the greenhouse on the last three hosts are also less severe than those on garden beet.

It is notable that the beet mosaic virus has a restricted host range except in the Chenopodiaceae, and occurs widely in nature on only a few wild hosts. It has been found to infect commonly zinnia and less commonly spinach, together with a strain of turnip virus 1 and a strain of cucumber virus 1. In the field, each of these three viruses produces symptoms on spinach that may easily be confused with those of each of the others.

The biennial nature of beet necessitates certain cultural practices if virus maladies are to be avoided in seed crops. Either the aphid vectors must be controlled during the overlapping period of the two seasons or steckling plants must be grown in sections free of viruliferous aphids. The cool prevailing temperatures of the Puget Sound section make it difficult to control aphid vectors sufficiently to prevent widespread virus infection. Consequently, it has been necessary to avoid the period during which viruliferous aphids are extremely migratory by growing steckling beds in sections isolated from seed fields. This practice, however, has not completely prevented the disease even though practical control has been obtained. Certain sections, even though excellently isolated, have been only moderately free of mosaic.

The symptoms, host range, and properties described for this virus indicate that it is the same virus that has been described as causing sugar-beet mosaic in this country (*5*, *7*, *8*, *12*). No differences could be seen in parallel inoculations with the Puget Sound virus and an isolate of beet mosaic virus from the Sacramento Valley of California. Properties and symptoms of the virus of sugar-beet mosaic in Idaho and California described by Hoggan (*7*) are almost identical with those given herein.

The beet mosaic in Europe (*1*, *2*, *4*, *6*, *13*, *14*, *15*, *16*) is thought to be caused by a virus distinctly different from that reported in this paper. The European virus has a much wider host range (*15*) than that of the beet mosaic virus described herein and distinctly different physical properties (*16*).

SUMMARY

A mosaic seriously affecting the beet seed crops in the Pacific Northwest is caused by a virus thought to be identical with the virus of sugar-beet mosaic previously reported from this country but different from the virus reported as causing beet mosaic in Europe.

The beet mosaic virus was found to infect all chenopodiaceous plants tested by mechanical inoculation. Of the species tested in other families the virus infected only *Verbena hybrida*, *Viola tricolor*, *Stellaria media*, *Tetragonia expansa*, *Aster amellus*, *Zinnia elegans*, *Amaranthus retroflexus*, *Capsella bursa-pastoris*, and *Iodanthus pinnatifidus*. The first 2 of these hosts were symptomless carriers. Over 50 species in 16 different families did not become infected upon mechanical inoculation. *Beta vulgaris*, *Spinacia oleracea*, *Zinnia elegans*, and *Amaranthus retroflexus* are considered to be the primary natural hosts.

Two insects, the black bean aphid and the green peach aphid, found commonly in beet fields in the Puget Sound section, are vectors. Transmission was also obtained with the cabbage aphid, but it is not

thought that this insect is a common vector. The virus is also readily transmitted by mechanical means. It was recovered from semi-mature but not from mature seed. Evidence presented indicates that it is not seed-borne.

A study of the physical properties of the virus showed it to be inactivated when aged in vitro for 72 hours at 20° C., when diluted 1 to 2,000, and when heated for 10 minutes at 61°.

Practical control of the disease was obtained in the Puget Sound section by growing steckling beds in sections well isolated from diseased seed fields.

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THE HEMICELLULOSES OF MAIZE COBS AND RYE STRAW[1]

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INTRODUCTION

The carbohydrates of the cereal straws and products represent a type of raw material that can be obtained in large amounts and that might be of great commercial value. Of these carbohydrates the most important are hemicelluloses and cellulose. The term "hemicellulose" includes polyoses, polyuronides, and cellulosans. Their chemical relationship to other constituents in the plant is not known definitely nor is the nature of their origin.

This report presents the results of a partial qualitative study of the sugars in the hemicelluloses of maize cobs and rye straw as well as a determination of their chain length and number of functional groups.

REVIEW OF THE LITERATURE

In 1930, Preece (*13*)[2] fractionated the hemicelluloses of maize cobs into four groups. The products of hydrolysis consisted principally of xylose, a methyl pentose, arabinose, and a uronic acid. One fraction corresponded to the formula

$$(C_5H_8O_4)_{43}\cdot(C_6H_{10}O_4)_8\cdot(C_6H_8O_6)_2$$

with a molecular weight of approximately 6,466. In 1936, Angell and Norris (*2*) found that the maximum yield of fraction A from this source depended upon the pH at which the precipitation was brought about; the isoelectric zone was found to be pH 4.0–4.1. These authors were unable to confirm the presence of the methyl pentose.

The hemicellulose of rye straw have been less well studied. Norman (*12*) indicates that they contain at least two fractions, A and B; the nature of the sugars present was not determined. Fraction A contained 5 percent of uronic acids, 60 percent of an anhydropentose, and 35 percent of an anhydrohexose; fraction B was made up of 29 percent of uronic anhydrides, 60 percent of anhydropentose, and 11 percent of anhydrohexose.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 46.

EXPERIMENTAL METHODS

White maize cobs and rye straw were both grown on college land which consisted of Merrimac fine sandy loam. The materials were ground to pass a 1-mm. sieve, and were freed of contaminating substances in the manner usually employed in such studies.

The hemicelluloses were removed from the prepared material by extractions with 4-percent sodium hydroxide. Four 22-hour extractions were made in the cold; a final extraction was made for 6 hours in a bath of boiling water. The extracts were mixed and the hemicelluloses precipitated completely by means of acetic acid and 95-percent alcohol, due attention being paid to the isoelectric points. No attempts were made to fractionate the precipitate. On the extracted basis, the yield was about 33 and 18 percent respectively. Purification was effected by the method of Anderson et al. (*1*) and by electrodialysis. The sodium sulfite test indicated the presence of a trace of lignin after purification.

The methods employed for characterization were those cited previously (*3*) and those now to be described. The iodine and alkali numbers and R_{Cu} values were determined by the methods of Kline and Acree (*10*), Schoch and Jensen (*14*), and Farley and Hixon (*6*) respectively. The iodine number, defined as the number of cubic centimeters of 0.1 N iodine in alkaline solution which will react with 1 gm. of carbohydrate material, is a measure of the aldehyde group (*4*). On the assumption that the aldehyde group is a terminal unit, the molecular weight of the carbohydrate may be calculated by the use of equation

$$RCHO+I_2+3NaOH\rightarrow RCOONa+2NaI+2H_2O \qquad (1)$$

By inspection it may be seen that 20,000 cc. of 0.1 N iodine will be necessary to oxidize the aldehyde group of 1 gm. molecular weight of the polysaccharide to the carboxyl group. Hence, the average molecular weight of heterogeneous polysaccharide may be calculated from the formula

$$20{,}000/\text{iodine number}=\text{molecular weight} \qquad (A)$$

In a manner similar to that described above, the R_{Cu} number may also be used to estimate the molecular weight (*7*). The values obtained, however, are extremely low because the reaction does not appear to be stoichiometric. A calculation by formula B (*11*) is a more suitable procedure.

$$132\times R_{Cu}\ (\text{xylose})\ /R_{Cu}\ (\text{hemicellulose})=\text{molecular weight} \qquad (B)$$

Analytical data not included here seem to indicate that the R_{Cu} for xylose is approximately 2,247.

Acetylations were conducted according to the method of Haworth and coworkers (*9*). In order to obtain a successful acetylation of hemicelluloses from rye straw, it was necessary to hydrate the material and then to dehydrate it without allowing it to become dry. Acetyl values were determined by the procedure of Bryant and Smith (*5*).

Specific rotations were calculated from data obtained from a Schmidt and Haensch saccharimeter.

Hydrolyses were effected by a 4-percent solution of sulfuric acid during about 4 hours' time in a boiling water bath. The ratio of sample to acid was 1 to 50.

ANALYTICAL DATA

A résumé of the data obtained is given in table 1. Fermentation tests on the hydrolytic products indicated the presence of a very small amount of hexose sugars. Gas was not produced until after 6 hours of incubation; no gas whatever was produced in the blanks for the same period of time. The saccharic and mucic acid test were not wholly conclusive. Mannose was not present. By means of benzylphenylhydrazine, *l*-arabinose was found in the hemicellulose from maize cobs, but not in that of rye straw. The presence of xylose was established by the xylonobromide test.

TABLE 1.—*A partial chemical analysis of hemicelluloses from maize cobs and eye straw on an ash- and moisture-free basis*

Determination	Hemicellulose	
	Maize cobs	Rye straw
Hexose	Trace	Trace
Uronic acid anhydride, percent	3.75	3.67
Anhydroxylose, percent	81.2	85.8
Arabinose	Trace	Not detected
Alkali number	11.98	11.78
Iodine number	3.43	1.14
Molecular weight	5,831	17,543
R_{Cu} value (milligrams of copper per gram of polysaccharide)	73.00	50.00
Molecular weight (from formula B)	4,063	5,932
Molar ratio of uronic acid anhydride to anhydroxylose	1:29	1:31
Molecular weight	4,004	4,268
Specific rotation $[\alpha]_D^{20}$		
Before hydrolysis, degrees	−93.8	−94.2
After hydrolysis, degrees	+35.1	+27.5
Acetyl value:		
Calculated (for xylan diacetate, $C_9H_{12}O_6$), percent	39.8	39.8
Found, percent	40.2	39.7

The residue after hydrolysis amounted to 2.5 and 0.7 percent respectively for maize cobs and rye straw. Only a trace of furfural was detected in the residue from the maize cobs. It need not necessarily be assumed that the reacting compound came from the sugars of unhydrolyzed hemicellulose. Preliminary tests on the rate and procedure of the hydrolysis indicated that a maximum of reducing power was obtained in 4 hours. The initial slope of the curves was similar to that obtained from starch when treated in the same way. The positive optical rotation of the hydrolyzed hemicellulose and the strong negative rotation prior to hydrolysis, together with the rate of hydrolysis, strongly suggest that a beta linkage is present and that a pyranoside structure is predominant. Haworth and coworkers (*8*) found that xylan from esparto cellulose of different origin contains xylopyranose residues and a constant proportion of combined *l*-arabinose in the furanose form.

It should be emphasized that the preparations used for the analyses were considered to be heterogeneous; hence the molecular weights reported represent the average or the mean of the different fractions. As indicated by the iodine number and the R_{Cu} values, the average molecular weights differ considerably. These variations for either product are due in part to differences in the sensitivity of the methods. The iodine method is believed to yield fairly accurate data for sub-

stances composed of relatively short chains (*10*). The values obtained by this method for rye straw hemicelluloses, however, appear to be too high.

The molecular weights determined from the ratio of uronic acid anhydride to anhydroxylose are in fair agreement with those obtained from formula B, and probably represent the more reliable data. The ratios refer to a chain consisting of 29 and 31 anhydroxylose units respectively, each having a hexuronic acid anhydride terminal unit. On this bases the molecular weights are approximately 4,004 and 4,268 respectively for the 30 and 32 unit chains. The hexose and arabinose are constituents of a chain, but their position and amount are not known; hence they have not been considered in these calculations. Anhydroxylose and a hexuronic acid anhydride appear to account for approximately 85 and 90 percent of the hemicelluloses of maize cobs and rye straw respectively.

The two hemicelluloses are similar to each other and to commercial cornstarch in their degree of alkali liability.

The carbon and hydrogen content of the acetate of maize-cob hemicellulose was 49.5 and 5.9 percent respectively. The theoretical percentage content of carbon and hydrogen in xylan diacetate, $C_9H_{12}O_6$, is 49.98 and 5.60 respectively.

The data presented strongly suggests that the hemicelluloses of maize cobs have a beta linkage and have the pyranose structure. The hemicellulose appears to consist of 81.2 percent of anhydroxylose, 3.75 percent of uronic acid anhydride, and a trace of both a hexose and arabinose. The hemicellulose of rye straw appears to possess the same type of linkage and structure and to consist of 85.8 percent of anhydroxylose, 3.67 percent uronic acid anhydride, and a trace of hexose; arabinose was not detected.

SUMMARY

The total hemicelluloses of maize cobs and rye straw were isolated and purified. Data for both strongly suggest that the beta linkage is present and that the pyranose structure is predominant. Determinations of the average molecular weights of the hemicelluloses by reducing methods and by calculations based on the ratio of uronic acid anhydride to anhydroxylose indicate a considerable variation, but the most probable values are believed to be between four and six thousand.

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